Published in final edited form as:

Methods Mol Biol. 2012 ; 757: 369–397. doi:10.1007/978-1-61779-166-6_22.

Overview-studying integrins in vivo

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Introduction

Integrins are expressed in all nucleated cells of multicellular animals and are essential for cell-matrix adhesion and, in vertebrates, cell-cell interactions. Since their recognition in 1987, they have been one of the most widely studied family of cell adhesion receptors. Integrins contribute to development, hemostasis, the immune response as well as diseases such as cancer and autoimmunity. In addition to their role as mechanical links, integrins are important conduits of bidirectional signaling in cells that influence processes from cytoskeletal arrangement and growth factor signaling to gene transcription (1). This overview will highlight a number of the major physiologic functions of integrins in both higher and lower organisms. Use of genetic approaches (i.e. gene knockouts) has provided a wealth of information about integrin function and will be emphasized in this chapter; given the significant published literature in this area we will focus on those examples in which specific functions have been revealed.

Integrin genes in vertebrates and invertebrates

Integrins are $\alpha\beta$ heterodimeric, transmembrane proteins that are restricted to metazoa. *Caenorhabditis elegans* (C-elegans) has one β and two α subunits forming two integrins, *Drosophila melanogaster* also has orthologs of these primordial integrins referred to as PS1 and PS2. These two specialize in adhesion to basement membrane laminins, and recognition of Arg-Gly-Asp (RGD) peptides present in extracellular molecules such as fibronectin and vitronectin, respectively (1). Mammalian integrin α subunits can be classified into four subfamilies (Figure 1). One group ($\alpha3$, $\alpha6$, and $\alpha7$), which is related to *Drosophila* PS1, pairs mainly with the $\beta1$ subunit to form the major laminin receptors in mammals (Table 1). Another subgroup ($\alpha1b$, αv , $\alpha5$ and $\alpha8$), which is related to the *Drosophila* PS2 proteins, pairs mainly with $\beta1$ and $\beta3$ subunits to form the primary receptors for RGD-containing extracellular matrix (ECM) proteins. In mammals, this group of integrins plays a particularly important role in early embryonic development. In mammals, the RGD motif is also present in non-ECM proteins (e.g. fibrinogen, latent TGF β), thus expanding the repertoire of ligands recognized by this integrin subfamily to allow development of novel cell-specific functions. This is evident in the PS2 subgroup whose members perform more specialized functions in bone, kidney and platelets. For example, $\alpha1b\beta3$ integrin on platelets recognizes a motif in fibrinogen similar to RGD to promote platelet adhesion. Although the PS1 and PS2 groups of integrins are present in many invertebrates, including C-elegans, the PS3-PS5 groups characterized in *Drosophila* seem to be specific for insects and may align with the laminin group when homology across the whole protein is compared. The third mammalian subgroup, the $\alpha4/\alpha9$ cluster is present only in chordates. These α subunits pair with $\beta1$ or $\beta7$ to form integrins that recognize a host of ECM proteins as well as certain plasma proteins,

cell-associated counter-receptors of the IgG superfamily such as VCAM-1 and vascular endothelial cell growth factors (2).

A structurally and functionally unique subgroup of integrins arose in chordates from the incorporation of an “inserted” domain or “ α I domain”, which is not found in invertebrates. This protein motif is also referred to as the “A” domain as it has structural similarity to a domain present in von Willebrand factor. The I (A) domain is present in proteins that are components of multiprotein complexes and promote cell adhesion frequently requiring divalent cations (3). The I domain contains a metal ion dependent adhesive site (MIDAS) that comprises the ligand binding domain of the integrin. Four of these integrins (α 1, α 2, α 10 and α 11, all of which pair with β 1) are collagen receptors while the remaining five (α D, α E, α L, α M and α X, which pair with β 1 and β 7) are integrins expressed on leukocytes. Counter-receptors for the leukocyte integrins are present mainly on vascular endothelial cells and include members of the IgG superfamily, (ICAMs and VCAM-1) as well as the unrelated protein E-cadherin. The leukocyte integrins also recognize plasma proteins such as complement component iC3b and fibrinogen.

The integrin β subunits can also be classified into three major phylogenetic branches (Figure 2). Two are represented in vertebrates (group A and B) while the third contains only invertebrate sequences. Group A contains β subunits β 1, β 2 and β 7 that are associated with most of the α -subunits from the PS1, PS2 and I domain subgroups. Of these, β 1 is the most widely expressed in many cell types, primarily because it associates with α 1- α 11 and α v subunits. The β 2 and β 7 subunits are restricted to hematopoietic cells. The β integrin subunits within Group B (β 3, β 4, β 5, β 6, and β 8 proteins) tend to have a more restricted tissue distribution and associate with fewer α chains (Table 1). It is important to note that the production of α and β chains within any given cell type may not be balanced; often the widely expressed subunits such as α v or β 1, which are promiscuous in their pairing with β and α subunits, respectively, are overproduced relative to other subunits. However, only the intact heterodimeric $\alpha\beta$ integrins are found on the cell surface – unpaired α or β chains are retained in the endoplasmic reticulum and degraded. This restricts the combination of α and β subunits and thus the integrin repertoire on the surface (2). The repertoire of integrins can switch at the cell surface as a result of gene transcription that is in some cases regulated by signaling events elicited by ligated integrins themselves. This in turn has effects on cell fate as distinct ECM components initiate integrin signaling events to promote proliferation versus differentiation (4). The fact that only specific α and β chains pair in defined patterns suggests that these chains must have co-evolved, even though the genes encoding them are very different and are distributed throughout the genome. This co-evolution must have occurred with the development of novel major functional systems in which the integrins are required; for example the development of the adaptive immune system necessitated co-evolution of α L, α M, α X and β 2 integrins to form the major receptors needed for guiding immune cells during host defense and inflammatory reactions (2).

Integrin signaling events – inside out and outside in

The ability of integrins to bind their ligands (whether ECM proteins or counter-receptors on other cell types) is dynamically regulated. This allows cells to carefully control their ability to adhere to matrix or establish connections with other cell types. This property is obviously important during cell migration in development and on circulating immune cells that need to migrate out of the vasculature at sites of infection or injury to mediate host defense. The processes regulating integrin affinity changes are referred to as “inside-out” signaling (Figure 3). Such events are best recognized for β 2 and β 3 integrins but are probably common to all integrin types. The leukocyte β 2 integrins and platelet integrin α IIb β 3, reside in a resting state (bent/closed conformation) and are rapidly activated (extended/open

conformation) to bind ligand when the cells that express these integrins receive activation signals. In addition to conformational changes, inside-out signals control integrin clustering and accumulation at specific regions of the plasma membrane. In leukocytes, these activating signals can be transduced by chemokine and other inflammatory receptors as well as receptors for members of the selectin family of adhesion molecules. In platelets, $\alpha\text{IIb}\beta 3$ activation is triggered by G-protein coupled receptors, by the von Willebrand factor receptor or by collagen receptors (such as GPVI) (1). Major insights into integrin activation have come from studies of integrin structure obtained by crystalization of the ligand binding A domains. Additionally, the identification of various intracellular signaling proteins that associate with the integrin cytoplasmic tails have helped define some of the steps in integrin activation during inside-out signaling responses (1). Although integrin activation is best described in circulating immune cells, as it serves to localize the accumulation of cells to sites of inflammation, it is likely that dynamic regulation of integrin affinity plays a central role in processes such as vascular development and neurite outgrowth (5).

Integrins are unique among transmembrane receptors in their ability to transmit bidirectional signals. Ligation of integrins by extracellular matrix proteins leads to the assembly of a number of cytoplasmic proteins that link the integrin to the actin and microtubule cytoskeletal network (Figure 3). Active integrins in turn participate in the regulation of the cytoskeleton and reorganization of the extracellular matrix. Ligation of integrins also leads to the transmission of intracellular signals that modulate many aspects of cell behavior. This includes stimulation of cell cycle via ERK and cyclin D1 and others, inhibition of apoptosis via PI3-kinase, Akt and NF κ B, changes in shape, polarity and motility via protein tyrosine kinases, phosphatases and members of the Ras and Rho family of small GTPases. The activation of these signaling pathways, which are referred to as “outside-in” signal transduction events, also leads to the modulation of gene expression (1). Anchorage dependent signaling through integrins is, in some cases, required for cellular responses to growth factors and other receptors. For example, in immune cells, $\alpha\text{L}\beta 2$ integrin mediated T cell adhesion to antigen presenting cells is required for induction of T-cell receptor-specific T cell responses (6).

Integrins in lower organisms

Integrins are widely expressed in embryonic tissue of *Drosophila* and *C. elegans* and studies in these organisms have defined the minimal set of integrins required for fundamental processes in development. The two integrins identified in *C. elegans* correspond to the *Drosophila* PS1 (laminin integrin) and PS2 (RGD integrin). The two alpha subunits, $\alpha\text{pat-1}$ and $\alpha\text{pat-2}$ pair with a single β subunit, $\beta\text{pat-3}$. *Drosophila* has 5 α -subunits PS1 through PS5, and two β subunits, βPS and Bv . βPS is most similar to both the *C. elegans* β subunit and β subunits found in vertebrates (7). The basement membranes in fruit flies are composed principally of four proteins, type IV collagen, laminin, nidogen/entactin and the heparan sulfate proteoglycan perlecan. These have homologues in vertebrates suggesting that they form the basis for an early basement membrane. Other well known vertebrate ECM proteins are absent including fibronectin, vitronectin, osteopontin, fibrinogen, and von Willebrand factor. These ECM proteins, important in vascular development and adhesion functions of blood cells, may have appeared later during the evolution of vertebrates (8).

Loss of function mutations of integrins or their extracellular matrix ligands in fruit flies and nematode worm leads to lethality that is attributed to defects in muscle attachment and contraction, cell migration, gut morphogenesis and adhesion between epithelial cell layers. Distinct roles for the RGD and laminin binding integrins have been documented in some of these cases. For example, RGD-binding integrins are required for muscle contraction by organizing the actin-myosin contractile structure into sarcomeres in *C. elegans*, and

attachment of the ends of the muscle to the ECM in *Drosophila*. Laminin binding integrins promote differentiation of endodermal cells required for gut morphogenesis, with integrin deletion leading to misshapen pharynx in the mutant *C.elegans* larvae, and midgut in *Drosophila* mutant larvae (7). These examples also illustrate the point that despite the large differences between these two simple model organisms, the role of integrin/extracellular matrix interactions for fundamental processes during development are conserved. Roles of both RGD and laminin binding integrins in post-embryonic development have been identified by generating organisms that are mosaic for homozygous mutant cells. *Drosophila* PS1 and PS2 are present in the developing wing which is made of two layers of epidermal cells with PS1 on the dorsal and PS2 on the ventral side. Both integrins are required for providing adhesion between the basal surfaces of the dorsal and ventral epithelia. The complementary expression of integrins may be required to insure asymmetric adhesion and thus prevent wrinkling due to adhesion within the same layer of cells (7). In addition to maintaining structural integrity in animal tissues, laminin integrin anchoring to the ECM in *Drosophila* is essential for establishing a stem cell niche that in turn is important for the retention of a stable stem cell population. This integrin mediated function, first established in *Drosophila*, has been shown to be conserved in vertebrates (9). Finally, studies in both flies and worms suggests that axon guidance is dependent on the laminin-binding integrins (10). The role of the other *Drosophila* integrins, PS3-PS5 are not well described. Mutations in PS3 affect short-term olfactory memory (11) while the function of PS4 and PS5 remains unknown.

Focal contacts link integrins to the cytoskeleton in mammalian systems and contain cytoskeletal/adaptor/linker and signal transduction molecules such as talin, α -actinin, vinculin, paxillin, FAK, ILK, p130 and others. These proteins are well conserved in *Drosophila* and possibly *C. elegans* (8). Genetic deletion of several of these proteins has revealed their relative importance in integrin dependent functions. For example, talin deficiency mimics integrin deficiency whereas deletion of ILK results in a much weaker defect (12). The conservation of integrins, their cytoskeletal associated proteins and extracellular matrix ligands in flies, worms and people makes these genetically tractable lower organisms attractive for analysis of ECM-integrin-cytoskeleton connections. Also the presence of the aforementioned signaling molecules as single genes in flies and worms reduces the possibility of redundancy and thus functional overlap in the system (8). However, it is also evident that vertebrates have evolved proteins with more specialized functions than those found in flies and worms. This is particularly evident in vascular biology, some aspects of neurobiology and likely other uniquely vertebrate functions requiring multicellular adhesive interactions. The advent of genetic engineering in mice has provided important insights into the specialized, non-redundant roles that integrins and their interacting proteins, have developed in higher organisms.

Integrins in mammalian development and pathophysiology

All the individual integrin subunits have now been deleted in mice and the reported phenotypes highlight the non-redundant, specialized functions that integrins play in vivo (13). Embryonic lethality upon genetic deletion of some of the integrins may have been predicted from the known expression pattern of the integrin during development. Additional information about the role of these integrins in adult mice will be undoubtedly forthcoming as more conditional knock-out animals are generated – we have seen this especially with $\beta 1$ integrins as outlined below. The phenotypes of knock-out mice lacking α or β subunits are grouped according to the organ system most effected by their deletion.

Vasculature

A number of integrins play central roles in vascular formation during fetal and embryonic development as well as regulating blood vessel integrity in adult animals (14, 15). This function is served through both binding of ECM proteins by vascular endothelial precursors (which provides “outside-in” signals to the cells to guide their migration during vascular branching) and through the ability of integrins to specifically bind a number of growth factors (in particular vascular endothelial growth factors – VEGFs) essential in blood vessel development. Integrin-mediated adhesion events also play a critical function in pathologic angiogenesis, for example in response to tumor development or chronic inflammation. Most of the integrins involved in vascular development and integrity are members of the PS2 family that recognize RGD peptide motifs in ECM proteins. Vasculogenesis is completely dependent on $\alpha 5\beta 1$ and its ligand fibronectin (16). Mutation of $\alpha 5$ leads to early embryonic lethality characterized by mesodermal defects and poor vascularization of both the yolk sac and the embryo itself (17). This phenotype is similar, but not as severe as that observed in mice lacking fibronectin (18). Mutation of $\beta 1$ has an even more profound effect, as expected due to the loss of many integrins, which manifests as gastrulation defects and pre-implantation mortality (19). Interestingly, the phenotype of Tie2-Cre; $\beta 1^{\text{flox/flox}}$ mutants, in which all $\beta 1$ -integrins are deleted in endothelial cells only, closely resembles the phenotype of $\alpha 5$ mutants, suggesting that endothelial-expressed $\alpha 5\beta 1$ is the dominant integrin that recognizes fibronectin during vascular morphogenesis (20). In contrast, during pathologic vascularization $\alpha \nu \beta 3$ integrin appears to play a central role. This integrin is highly expressed in blood vessels at sites of tumor growth and wound healing (21). Indeed, treatment of animals with anti- $\alpha \nu \beta 3$ mAbs blocks neovascularization in tumor models, (22). In this role it appears that it is the ability of $\alpha \nu \beta 3$ to bind VEGF and even to associate with the VEGF receptor that mediates its role in pathogenic neovascularization. In tumor cells, expression of $\alpha \nu \beta 3$ correlates with overproduction of VEGF (23) while, probably more importantly, in endothelial cells $\alpha \nu \beta 3$ directly associates, and synergizes with VEGF receptor signaling to promote enhanced responses to this growth factor (24, 25).

The physiologic association of integrins with vascular growth factors is also revealed in the phenotype of $\alpha 9$ mutant mice. Surprisingly, deficiency of $\alpha 9$ produces a lymphatic vascular defect that results in disordered lymphatic development and perinatal lethality due to accumulation of lymph fluid in the thorax (26). Lineage specific ablation of $\alpha 9$ in blood vessels (using $\alpha 9^{\text{flox/flox}}$; VE-cadherin-Cre mice) revealed that $\alpha 9\beta 1$ plays a critical role in assembly of fibronectin bundles in the valves of developing lymphatic vessels, which is required to prevent leakage of lymph fluid (27). However, integrin $\alpha 9\beta 1$ is also the major binding protein for VEGF-C and VEGF-D, which together are the two primary growth factors for lymphatic vessels (28). Hence, much of the lymphatic phenotype of $\alpha 9$ deficient mice can be ascribed to loss of VEGF-C and VEGF-D signaling events in the lymphatic precursors.

Deletion of another major group of integrins, those containing the $\alpha \nu$ subunit, reveals the importance of integrins in organizing the parenchyma surrounding the vasculature. Mice lacking $\alpha \nu$ are embryonic lethal due to widespread vascular malformations and hemorrhage. However these arise later in development (after e10.5) and affect primarily the brain in the embryo (29) due to defective interactions of cerebral vessels with surrounding brain parenchyma (30). The specific deletion of $\alpha \nu$ in endothelium has no effect on vascular development, which is consistent with its relative absence in this microvasculature, whereas its deletion in neural cells, particularly glia leads to cerebral hemorrhage (31). Of the $\alpha \nu$ integrins, it appears that $\alpha \nu \beta 8$ is the primary heterodimer involved in this process, since mice lacking $\beta 8$ also have cranial vascular defects and intracerebral hemorrhage (32). Indeed, targeted deletion of $\beta 8$ within neuroepithelium ($\beta 8^{\text{flox/flox}}$; nestin-Cre mice) is sufficient to produce the late developmental cranial hemorrhage phenotype (33). Disruption

of $\alpha 4$ also has a later vascular phenotype resulting from failed fusion of the allantois with the chorion during placentation, as well as impairments in cardiac development (34, 35). In this case the ligand is likely VCAM-1, as mice lacking this member of the IgG superfamily exhibit a similar phenotype to the $\alpha 4$ deficient mice (36).

Ocular/CNS

A number of studies have demonstrated the critical function of integrins in ocular and CNS development and hemostasis. Within the eye, lens development is best studied, since this tissue consists of relatively simple assemblies of fibronectin, collagen and laminin (37). Integrins that recognize these ECM proteins have all been implicated. Of them, clearly the $\beta 1$ containing heterodimers are most important, since mice lacking $\beta 1$ specifically in the lens ($\beta 1^{\text{flox/flox}}$; MLR10-Cre animals) are microphthalmic due to apoptosis of the lens epithelium and disintegration of the lens fibers (38). However, whether this major defect is due to loss of fibronectin receptors (such as $\alpha 5\beta 1$) versus collagen receptors ($\alpha 1\beta 1$) or laminin receptors ($\alpha 3\beta 1$) remains to be seen and will only be sorted out using additional lineage specific mutagenesis methods. In lens development, there is also clear evidence of compensation by different integrins within the same class. For example, absence of the $\alpha 3$ or $\alpha 6$ integrins (which when paired with $\beta 1$ form the major laminin receptors in the developing lense) by themselves have little effect on lens development. In contrast, $\alpha 3/\alpha 6$ double mutant mice show severe dysmorphogenesis of the developing lens (39).

The involvement of integrins in retinal development more closely mirrors their overall contribution to CNS formation, mainly as related to their role in guiding the migration of neuronal precursor cells (40). Though early studies (using blocking mAbs) had suggested members of the $\beta 1$ integrins play a dominant role in guiding neuronal migration along glial fibers in the developing brain, more modern approaches with conditionally deficient mice have revealed more subtle functions. Specifically mice lacking the $\beta 1$ subunit in neurons only ($\beta 1^{\text{flox/flox}}$; nestin-Cre animals) actually show relatively normal neuronal cell migration but have a completely disorganized cortex as a result of a defect in association between cortical structures and the developing meningeal basement membrane (41). This phenotype resembles the CNS cortical defects seen in mice lacking the $\alpha 2$ chain of laminin (42), suggesting that a laminin $\beta 1$ integrin receptor (such as those in the PS1 family) is involved. Integrins and other cell adhesive molecules also play a major role in stabilizing neuronal synapses (43). Loss of this stabilizing function, as evidenced primarily by $\beta 3$ integrin blocking studies, results in neuronal dysfunction that manifest as defects in long-term potentiation necessary for learning and memory (40). It is likely that other axonal guidance molecules, such as the netrins, semaphorins, and ephrins play the dominant role in guiding neuron migration during development (44).

In contrast to these findings in the CNS, there is better evidence for the involvement of integrins (in particular $\beta 1$ family members) in neural crest migration during development of the peripheral nervous system. Mice with conditional deletion of $\beta 1$ integrins in neural crest cells ($\beta 1^{\text{flox/flox}}$; Ht-PA-Cre animals) exhibit severe perturbations of the peripheral nervous system, including failure of normal nerve arborization, delay in Schwann cell migration, and defective neuromuscular junction differentiation, all of which can be attributed to defective migration of these cells through the embryonic ECM (45). The $\beta 1$ integrins also directly affect peripheral nervous system Schwann cells, as conditional deletion of $\beta 1$ in these cells ($\beta 1^{\text{flox/flox}}$; P₀-Cre mice) results in reduced survival, proliferation and differentiation of Schwann cell precursors (46).

The RGD binding integrin $\alpha \nu \beta 5$ plays a critical role in maintenance of retinal pigment epithelial cells in the eye. Mice lacking the $\beta 5$ integrin slowly develop age related blindness due to progressive accumulation of shed pigment from retinal cells. The retinal pigment

epithelial cells use the $\alpha v\beta 5$ integrin as a phagocytic receptor to mediate uptake of shed photoreceptor particles, which occurs as a natural diurnal process in the rods and cones of the retina (47).

Integrins clearly play a role in a number of neuroinflammatory diseases, such as multiple sclerosis. This likely reflects their major function in regulating immune cell trafficking in the body. There is also suggestive evidence that neuronal survival in degenerative diseases such as Parkinson's disease or Alzheimer's disease may be influenced by integrin signaling (44). This has raised interest in integrin targeted therapeutics as a means to treat neurologic disease.

Immune

The contribution of integrins to immune system (and general hematologic) function has been extensively studied and in fact serves as the paradigm for our understanding of many facets of integrin biology. The involvement of integrins in regulating neutrophil, monocyte and lymphocyte trafficking through out the immune system has been well studied both *in vitro* and more recently *in vivo* using a variety of intravital microscopy methods. Furthermore, the overall structural changes that take place during integrin activation have been best characterized in the leukocyte and platelet integrins, allowing a direct, but as yet incomplete understanding of how the protein associations involved in the "inside-out" signaling response mediate integrin subunit unfolding. The primary integrins expressed by immune cells are $\alpha 4\beta 1$, $\alpha 5\beta 1$, all of the $\beta 2$ integrins, $\alpha v\beta 3$ and $\alpha E\beta 7$. The primary platelet integrin $\alpha IIb\beta 3$ is also found on some immune cells.

The most well appreciated role for integrins in the immune system is their function in guiding leukocyte exit from the vasculature into the tissues, either within lymph nodes or directly into sites of tissue injury or inflammation (Figure 4). The process of leukocyte diapedesis from the vasculature is termed the leukocyte adhesion cascade (48). Broadly, this cascade involves three general classes of molecules – the selectins, chemokines and integrins. The selectins are carbohydrate recognizing receptors, present both on the leukocyte and vascular endothelium, that allow initial tethering but not full adhesion of the blood cells to endothelial cells. As a result, selectin interactions result in rolling of the leukocyte along the vascular wall. During this process, the leukocytes are exposed to chemokines and other inflammatory mediators, which induces "inside-out" signaling responses leading to integrin affinity modulation and binding to ligands on the surface of activated endothelial cells. This promotes cell arrest. The regional chemokines facilitate the directed, local egress of these cells into tissues. This simple three step model – rolling mediated by selectins, activation mediated by chemokines and full arrest mediated by integrins – is an oversimplification, as there are examples of leukocyte integrins (such as $\alpha 4\beta 1$) that can mediate rolling (49) or which reduce the velocity of selectin dependent rolling. Similarly, there are many additional steps following firm adhesion, such as adhesion strengthening and signaling events (50) leading to leukocyte shape change that are mediated by integrins. Despite this complexity, the overall role of integrins in guiding leukocyte trafficking out of the vasculature is extremely well supported by the consequences of deficiency of leukocyte integrins. Mice deficient in $\beta 2$ show profound defects in migration of all leukocyte types, which results in profound T-cell defects, impaired neutrophil recruitment in response to infection and resulting immunodeficiency (51). As expected, these mice show significantly reduced responses to a number of inflammatory stimuli and are protected from immune mediated tissue damage in a number of disease models, such as cardiac reperfusion injury (52). The susceptibility these mice manifest to infection is similar to that seen in human patients lacking the $\beta 2$ integrin – a disease referred to as Leukocyte Adhesion Deficiency syndrome (discussed below). Lack of $\beta 1$ integrin on blood cells effects their migratory capacity, resulting in a failure of fetal hematopoietic stem cells to colonize

the fetal liver or spleen during development (53). In mature cells, $\beta 1$ plays a critical role in guiding lymphocyte migration to the skin, lung, peritoneum and liver, all primarily through the actions of $\alpha 4\beta 1$ (54). Finally, lack of the $\beta 3$ integrin results primarily in a loss of platelet function (with subsequent hemorrhage), mainly through absence of the $\alpha IIb\beta 3$ family member (55). This phenotype also mirrors what is seen in humans lacking this integrin, which develop a disease referred to as Glanzmann's thrombasthenia (discussed below).

Though there is clear evidence that integrins play a role in tissue specific recruitment of immune cells during inflammatory or disease processes – for example the specific involvement of $\alpha E\beta 7$ integrin in the migration of T-cells to epithelial tissues such as the skin and gut (56) – it is too simplistic to assume that integrins alone play the dominant role in all immune cell trafficking. Indeed, the relative role of integrins in leukocyte migration in some inflammatory responses may be quite minor. This extremely surprising suggestion has emanated from studies of mice lacking ALL the major leukocytes integrins -- generated by conditional and inducible deletion of $\beta 1$, αV combined with complete deletion of $\beta 2$ and $\beta 7$ in adult mice (57). Dendritic cells lacking all these adhesion molecules migrate normally into lymph nodes where they present antigens to stimulate immune responses. This indicates that migration through interstitial spaces may be less dependent on integrins than was originally envisioned and instead these molecules may have a dominant role only in allowing leukocyte exit from the vasculature. Whether this general conclusion applies to other immune cells besides dendritic cells remains to be examined. However, it is provocative studies such as this that continue to redefine our understanding of the role of integrins in the immune system.

Beyond their role in guiding cell migration, it is also clear that leukocyte integrins play a central role in activating the effector functions of immune cells through “outside-in” signaling (50). Often this signaling works with other receptors engaged on the cell. For example, co-stimulation of T-cells through their antigen receptors (TCR) and integrins (for example, when the cells are plated on a matrix-coated surface) leads to much more robust proliferative responses that stimulation through the TCR alone (58). Similarly, chemokine activation of neutrophils is dramatically enhanced in cells that are also stimulated through their integrin receptors (59). In some cases, the activation function of the leukocyte integrin is relatively more important than its homing/migration function. For example, in a model of thrombohemorrhagic vasculitis caused by endotoxin and cytokine exposure, deficiency of $\alpha M\beta 2$ integrin does not affect the ability of leukocytes to be recruited to the site of vascular inflammation, but once there the cells cease to become activated because they do not recognize complement deposits that form along the vessel walls. As a result $\alpha M\beta 2$ deficient neutrophils fail to release proteases that otherwise would cause vascular damage and hemorrhage (60). This clear role for “outside-in” signaling events is also seen in other physiologic conditions, such as keratinocyte development as outlined below.

Cutaneous

A number of integrins play a key role in development and maintenance of epidermal structures. Their primary function, along with other adhesion molecules, is to link keratinocytes to the underlying basement membrane (61). The primary integrins expressed in the epidermis are the laminin receptors ($\alpha 6\beta 4$ and $\alpha 3\beta 1$), the collagen receptor ($\alpha 2\beta 1$) and less abundantly the vitronectin receptor $\alpha v\beta 5$ (62). Most of these integrins are localized in focal adhesion contact sites in keratinocytes, where they link the extracellular matrix to the actin cytoskeleton of the keratinocyte to provide firm adhesion. The $\alpha 6\beta 4$ integrin is localized to hemidesmosomes that help link keratinocytes to each other directly.

As in other organ systems, the role these individual integrins play in epidermal biology is best revealed in knockout mouse models. Conditional deletion of all $\beta 1$ integrins in the

epidermis ($\beta 1^{\text{flox/flox}}$; Keratin5 or Keratin14-Cre mice) leads to incomplete perinatal mortality due to separation of the epidermis from the underlying dermis (63, 64). Some mice survive up to 6 weeks after birth but show an absence of hair follicles, poor keratinocyte proliferation with fibrous dermal deposition associated with chronic skin inflammation. Interestingly, deletion of the $\alpha 2\beta 1$ integrin or the $\alpha 3\beta 1$ integrin alone (through deletion of the α subunits) has very little effect on skin biology – the loss of $\alpha 3$ produces mild foot blisters – suggesting that the epidermal function of these receptors is largely redundant (65, 66). Most interesting, however, is when the $\beta 1$ integrin is deleted in adult epidermis, through use of an inducible K14-cre strain. In this case, if the skin is already formed, loss of $\beta 1$ integrins results in an enhanced proliferative responses of keratinocytes to spontaneous wounding. In addition, the number of melanocytes in the interfollicular epidermis is greatly increased (62). This observation highlights the role of integrin “outside-in” signaling in regulating keratinocyte growth, in this case in a suppressive fashion. Consistent with these observations, knockdown of $\beta 1$ integrins in mouse keratinocytes in culture leads to increased proliferation and differentiation (67). These results also illuminate the issue of secondary effects of these deletions, such as the cutaneous inflammation that occurs in newborn mice lacking epidermal $\beta 1$ integrins, which may complicate interpretations of primary effects on the skin. Such is the case in mice lacking the $\alpha 6\beta 4$ integrin, which develop epidermal and dermal separation, similar to that observed in humans born with $\beta 4$ deficiency (referred to as junctional epidermolysis bullosa syndrome, see below). In this case alterations in TGF β responsiveness in the absence of these integrins may contribute to inflammatory responses in the skin that contribute to the epidermal/dermal separation. However, unlike the $\beta 1$ integrins, it seems that $\alpha 6\beta 4$ does not directly regulate keratinocyte differentiation (68).

Pulmonary

In terms of development, many of the same integrins that contribute to cutaneous epithelial cell morphogenesis are involved in formation of the pulmonary epithelial layer. This is particularly true for the $\alpha 3\beta 1$ laminin receptor. Integrin $\alpha 3$ deficient mice die at birth due to renal and lung defects associated with disorganized subepithelial basement lamina and poor adhesion of epithelia to the basement lamina (69). This phenotype is in a sense a more profound manifestation of epithelial dysfunction than the modest skin blistering in these animals. Combinatorial mutants that more broadly affect laminin binding, such as double mutant $\alpha 3/\alpha 6$ mice have even a more severe epithelial phenotype that results in disordered limb development and severe pulmonary hypoplasia (39).

However, it is loss of the RGD binding integrin functions, in particular that of $\alpha \nu \beta 6$ and to a lesser extent $\alpha \nu \beta 5$ that result in the progressive pulmonary abnormalities, which have taught us the most about the intersection of integrins and TGF β regulation. The $\alpha \nu \beta 6$ integrin is expressed primarily in epithelial cells of lung, skin and kidney where it recognizes both fibronectin, but more importantly the latency associated peptide (LAP) that is non-covalently associated with newly secreted TGF β and keeps TGF β from binding its receptor (70). The LAP of both TGF $\beta 1$ and TGF $\beta 3$ has an RGD binding motif, which is recognized by $\alpha \nu \beta 6$ and $\alpha \nu \beta 8$. When these integrins bind the TGF β LAP, they cause dissociation of LAP/TGF β complex allowing TGF β to interact with its receptors present on epithelial cells and resident pulmonary macrophages. Binding of TGF β serves as a powerful anti-inflammatory stimulus in both cell types – the presence of activated TGF $\beta 6$ in the lung helps suppress inappropriate alveolar macrophage activation that would lead to lung injury (71). This amazing biology was revealed by study of the $\beta 6$ knockout mice. These animals develop progressive pulmonary (and skin) inflammation that is strikingly similar to that seen in TGF β mutant mice themselves (72). By age 6 months, $\beta 6$ deficient mice develop emphysema, which is due to overproduction of the alveolar macrophage protease MMP12, as a result of the chronic inflammation present in these mice (73). Re-expression of either $\beta 6$

or an activated version of TGF β as a transgene in alveolar and bronchiolar epithelial cells of $\beta 6$ mutants, rescues the pulmonary inflammation (73). Integrin regulation of TGF β is also essential in limiting MMP12 release after bleomycin or irradiation mediated lung damage (74). These phenotypes point to the importance of $\alpha v\beta 6$ mediated TGF β activation in the lung to restrict the inflammatory properties of resident alveolar macrophages. Interestingly, this modulatory effect of TGF β on macrophages also affects their phagocytic function. Alveolar macrophages play a major role in recycling pulmonary surfactant, however this function is lost in both $\beta 6$ and TGF β deficient mice, which is manifest as a progressive accumulation of surfactant material in addition to the inflammation that these mice develop (75). Hence, as seen in other macrophage types, the transition of alveolar macrophages to the inflammatory state (leading to release of MMP12 and other mediators) correlates with the loss of the ability to phagocytose and degrade normal resident proteins.

Pulmonary activation of TGF β is also mediated by $\alpha v\beta 8$ integrin. This may play more of an effect on regulating inflammatory and proliferative responses of airway epithelial cells, versus macrophages, as determined in cell or organ culture models using functional blocking $\beta 8$ mAbs (76). Complete loss of $\beta 8$ results in embryonic lethality due to early vascular malformations; a function that is likely not related to the ability of this integrin to activate TGF β (32).

Studies of $\alpha v\beta 5$ in pulmonary vascular permeability during lung injury have also revealed novel functions for this integrin in the lung. Mice lacking this integrin (or normal animals treated with a $\beta 5$ blocking mAb) manifest significant reduction in pulmonary edema and vascular permeability following either ischemia-reperfusion or hyperventilation (77). The effect was traced to the ability of $\alpha v\beta 5$ to bind and activate VEGF, which in turn directly stimulated the vascular permeability. Indeed, direct treatment of pulmonary epithelial cells with VEGF will induce actin stress fiber formation and cell retraction – a phenotype not observed in $\alpha v\beta 5$ mutant cells. Hence, as in vascular development, the ability of integrins to bind and activate vascular growth factors reflects an important function beyond just cell adhesion.

Musculoskeletal

As in the fruit fly and the worm, a number of RGD, collagen and laminin binding integrins serve critical roles in formation of muscle structures. In developing myofibrils, organization of integrin subunits into distinct focal adhesion sites in the sarcolemma allows for nucleation of actin filaments that help organize the actin/myosin structures (78). However, the individual integrins that contribute to this phenotype are less well defined, since loss of specific collagen receptors ($\alpha 1\beta 1$ or $\alpha 2\beta 1$) result in mild musculoskeletal defects. In addition to its role in vascular development, the RGD-binding integrin $\alpha 5\beta 1$ also plays a role in maintenance of muscle structure, as determined by examination of embryonic chimeric mice containing cells derived from both normal and $\alpha 5$ deficient stem cells. These mice develop a progressive skeletal muscular dystrophy phenotype, characterized by diffuse muscle cell degeneration and apoptosis (79). This phenotype seems not to be due to a developmental disorganization of the actin/myosin structure of the muscle cell, but instead to a loss of adhesive signaling between muscle cells and the ECM, which results in poor myoblast survival over time. This illustrates another well-accepted role for integrins in promoting cell survival. Indeed disruption of integrin mediated anchorage to the ECM leads to apoptosis, a process referred to as “anoikis”. Integrin “outside-in” signaling to the PI3K-Akt pathway may be a major regulator of anoikis, with a breakdown of this process in epithelial cells contributing to neoplasia and metastasis (80).

Of the laminin binding integrins $\alpha 7\beta 1$ clearly has the dominant role in orchestrating normal muscle development. Dystrophin, utrophin, and $\alpha 7\beta 1$ are the major laminin receptors in

skeletal muscle. Each of these receptors links laminin in the extracellular matrix and basal lamina to the myocyte actin cytoskeletal network (81). Loss of dystrophin produces human Duchenne's muscular dystrophy, which is characterized by progressive myocyte degeneration that leads eventually to mortality. Mutations in $\alpha 7$ also produce myopathy in humans, though not nearly as severe as Duchenne's dystrophy (82). Mice that lack $\alpha 7$ integrin also develop myopathy, characterized by poor force transmission, compliance and viscoelasticity in diaphragm muscle (83, 84). In addition, $\alpha 7\beta 1$ plays an important role in maintaining the neuromuscular junctions and muscle-tendon junctions as these structures specifically degenerate in the $\alpha 7$ deficient mice (85). Interestingly, in both Duchenne's patients and the mouse model of this disease (the *mdx* mouse), the levels of integrin $\alpha 7\beta 1$ increase 2 – 3 fold, suggesting that these laminin receptors can functionally compensate for each other. Indeed, in combinatorial mice that lack dystrophin and $\alpha 7$ or utrophin and $\alpha 7$, the myopathy phenotype is dramatically accelerated (86)(87). These observations clearly indicate that these laminin binding receptors are dispensable for muscle development, but play a critical role in maintaining the structural integrity and functional capacity of skeletal muscle, primarily by linking myocytes to the ECM.

A number of both RGD and collagen binding integrins play major roles in skeletal development and homeostasis. The most extensively studied is the integrin $\alpha v\beta 3$, which is abundantly expressed in osteoclasts and plays a central role in the ability of these cells to adhere to bone matrix and regulate bone morphogenesis. In normal bone, the primary RGD containing ligands recognized by osteoclast expressed $\alpha v\beta 3$ are osteopontin and bone sialoprotein (88). Adhesion of osteoclasts to these surfaces through $\alpha v\beta 3$ causes actin cytoskeletal rearrangement in the osteoclast, spreading and activates the cell to release proteases into a defined vacuolar space between the osteoclast and the bone, thus causing bone resorption and remodeling. In mice lacking the $\beta 3$ integrin, the loss of osteoclast function results in bone overgrowth (osteopetrosis), however in humans lacking $\beta 3$ (ie those with Glanzmann's thrombasthenia) bone density is unaffected. This may be due to increased expression of compensating $\beta 1$ integrins in the human patients (89). In the mouse, it is also clear that loss of the "outside-in" signaling events that are elicited by engagement of osteoclast $\alpha v\beta 3$ produce the functional equivalent of loss of the receptor. Hence deficiency of members of the Src-family of tyrosine kinases, Syk kinase, or downstream adapter proteins such as DAP12, or SLP76 or a guanine exchange factor for Rac GTPases, Vav3 all result in varying degrees of osteoclast dysfunction and, in the case of Src kinase mutant mice, severe osteopetrosis resulting in lethality (88, 90, 91).

The collagen binding integrins $\alpha 10\beta 1$ and $\alpha 11\beta 1$ also play specific, and rather limited, functional roles in musculoskeletal development. Mice lacking $\alpha 10$ develop growth retardation of the long bones in the limbs due to disorganized cartilage formation particularly at the growth plates (92). The chondrocytes in these mice manifest a disordered columnar arrangement, an abnormal shape and reduced proliferative capacity. As a result the collagen fibrillar network in the growth plates of $\alpha 10$ deficient animals is disorganized, leading to the growth plate failure and shortened bones. Integrin $\alpha 11$ plays a specific role in dental development through its regulation of periodontal ligament fibroblasts. The $\alpha 11$ deficient mice show severe defects in incisor formation characterized by disorganized periodontal ligaments leading to a block in tooth eruption (93). Embryonic fibroblasts from the $\alpha 11$ deficient mice show reduced cell adhesion and spreading on collagen I as well as reduced cell proliferation. This suggests that $\alpha 11\beta 1$ is specifically required on periodontal ligament fibroblasts for cell migration and collagen reorganization to help generate the forces needed for axial tooth movement.

Renal

A number of $\beta 1$ integrins have been implicated as having specific roles in renal development and kidney disease. These functions range from roles in the development of the entire kidney to specific effects on the epithelial structures within the collecting system or in responses of the glomerulus to injury. Studies of $\alpha 8\beta 1$ have revealed its unique role in renal development. Loss of $\alpha 8$ results in a high frequency of complete renal agenesis due to a failure of epithelial-mesenchymal interactions; epithelial-type cells referred to as ureteric bud cells (UB) fail to invade into surrounding mesenchyme to form the metanephric kidney (94). Although $\alpha 8\beta 1$ recognizes a number of RGD containing proteins, such as fibronectin and tenascin C, in fact the ligand present in the developing kidney which activates $\alpha 8$ function is a novel ECM protein referred to as nephronectin (95). Nephronectin is expressed by the invading UB cells and induces expression of $\alpha 8\beta 1$ in the surrounding mesenchymal cells. Indeed, genetic disruption of nephronectin results in the same type of renal agenesis as seen in $\alpha 8$ mutant animals (96). In this system, it is clear that the nephronectin/ $\alpha 8\beta 1$ interactions not only provide adhesive structures to guide invading kidney epithelia, but also are involved in induction of growth factors, specifically glial cell line-derived neurotrophic factor (GDNF), a member of the TGF β superfamily. GDNF binds to its receptor, the RET tyrosine kinase on the epithelium, to provide growth and proliferative signals to the developing renal epithelial cells (97). Loss of any of these molecules also produces a renal agenesis phenotype similar to $\alpha 8\beta 1$ deficiency. This system of interactions of different cell types in the developing kidney highlights the importance of integrin/ECM interactions in controlling organ morphogenesis by regulating cell growth, migration and adhesion.

Integrin $\alpha 3\beta 1$ also has a dominant role in renal development. Mice lacking the $\alpha 3$ subunit have a reduced number of collecting ducts in the renal papilla suggesting that this integrin plays a role in the subsequent steps of branching morphogenesis to form the renal tubular collecting system (69). When $\alpha 3$ is specifically deleted in the UB cells ($\alpha 3^{\text{flox/flox}}$; HoxB7-Cre mice), the kidney papillae are either absent or abnormal (98). As in the earlier steps of renal development, it is clear that $\alpha 3$ interactions in the developing papilla also induce expression of growth factors required for organogenesis. In this case, elaboration of the growth factor Wnt7B following $\alpha 3$ engagement helps regulate epithelial cell survival in the developing tubules to allow formation of the mature renal papilla.

Beyond $\alpha 3\beta 1$ and $\alpha 8\beta 1$ it is likely that other laminin, collagen and RGD binding integrins play supportive roles in kidney development. This conclusion derives from deletion of $\beta 1$ specifically in the UB cells ($\beta 1^{\text{flox/flox}}$; HoxB7-Cre mice). In this case, a severe defect in branching morphogenesis is observed, worse than just the $\alpha 8$ effect alone (99). Interestingly, when $\beta 1$ is lost later in renal development ($\beta 1^{\text{flox/flox}}$; Aqp2-Cre mice) kidney formation is normal, however the mutant animals develop more severe injury and poor collecting duct healing following ureteric obstruction. In this case, it is likely that the injured renal collecting tubular cells fail to produce GDNF and other growth factors that would support repair of kidney structures following injury (99). Again, this highlights the cooperation of integrins in growth factor responses within the kidney.

It is also clear that $\beta 1$ integrins play important roles in the development and maintenance of the glomerular structures of the kidney. In the glomerulus, the interactions between mesangial cells and podocytes with the glomerular basement membrane present in the fine vascular capillaries of the glomerulus forms the basic filtration structure of the kidney. These interactions are dependent on $\beta 1$ integrins, since specific depletion of $\beta 1$ in podocytes ($\beta 1^{\text{flox/flox}}$; podocin-Cre mice) leads to severe malformations of the glomerular basement membrane and effacement of podocyte/membrane contacts (100). As a result, the mice are born with massive proteinuria and rapidly progress to death. In the normal glomerulus, continued expression of $\beta 1$ integrins is important in maintaining the filtration function of the

kidney. During glomerular injury, for example as a result of immune complex deposition, $\beta 1$ integrins of all types are induced in mesangial cells and podocytes (101). These interactions lead to elaboration of growth factors that can contribute to mesangial cell proliferation during glomerular injury. Indeed, mice lacking $\alpha 1\beta 1$ integrin show protection from glomerular injury and reduced mesangial cell proliferation in a mouse model of Alport's syndrome (102).

Human integrin deficiencies

Clear support for the importance of integrins and their activation have come from "experiments of nature". Human mutations in integrins within 3 of the 5 mammalian subgroups have been shown to cause disease (103). These include mutations in either the α or β subunits of integrins $\alpha 6\beta 4$ (PS1 cluster, laminin receptors) or $\alpha IIb\beta 3$ (PS2 cluster, RGD receptors) or in the $\beta 2$ integrin subunit (with the I-domain containing leukocyte receptors). In the case of the $\beta 2$ and $\beta 3$ mutations, multiple integrins are affected, which contributes to the variability in the disease phenotype from patient to patient.

Reduced or absent expression of $\alpha 6\beta 4$ leads to the syndrome of junctional Epidermolysis Bullosa (EB) which presents as extreme blistering and fragility of the skin. The skin blistering is caused by the inability of epidermal cells to adhere to basement membranes present in the dermis, leading to epidermal/dermal separation. The EB syndrome is accompanied by an occlusion of the intestine, termed pyloric atresia, which is often postnatally lethal. The occluded oesophageal, urethral and pyloric tissues may result from the detachment and subsequent fusion of epithelial linings (103, 104). Mice lacking either $\alpha 6$ or $\beta 4$ have a very similar epidermal phenotype and suffer perinatal lethality.

Genetic mutations in αIIb or $\beta 3$ produce a syndrome referred to as Glanzmann's Thrombasthenia. These patients present with a range of problems, the most serious of which is a severe bleeding disorder due to platelet adhesion defects (105). Individuals with $\beta 3$ mutations will often have bone cysts or other skeletal abnormalities, likely due to loss of $\alpha v\beta 3$ function. Mutations in $\beta 3$ can lead to little synthesis of the protein or allow full expression but no activity of integrin. The mice lacking $\beta 3$ or αIIb serve as good models of Glanzmann's thrombasthenia (106).

Leukocyte adhesion deficiency I (LADI) syndrome is caused by genetic mutations within the $\beta 2$ subunit, resulting primarily in deletion or reduction in surface expression of multiple leukocyte integrins or in some cases, leading to near normal levels of integrins but defects in integrin function due to mutations in the ligand binding domain. These patients present with a variety of immune deficits, ranging from life threatening soft tissue infections, to wound healing defects and variable forms of infectious gingivitis. The classical clinical hallmark of LAD is the presence of tissue infections without formation of pus. This is due to the fact that impairment of $\beta 2$ expression leads to reduced leukocyte recruitment and migration to sites of tissue infection because immune cells are unable to adhere to vascular endothelium. All mutations are found in the $\beta 2$ subunit gene located on chromosome 21. Mutations that effect CD18 expression at the surface can lead to <0.3% to 2.5–31% $\beta 2$ integrin expression, which correlates with presentation of severe to more moderate disease, respectively. Point mutations within both the ligand binding domain leading to near normal surface expression but poor ligand recognition have also been described {Roos, 2001 #122}. Mice with $\beta 2$ deficiency also manifest a variety of immune defects due to poor leukocyte adhesion and hence are good models of LAD {Kakkar, 2004 #57}.

Leukocyte adhesion deficiency syndrome can also result from mutations in proteins involved in the inside-out pathway of integrin activation. Recent studies have identified a set of patients with bleeding, immune cell accumulation defects and an osteopetrosis-like bone

defect, that may result from failure to activate $\beta 1$, $\beta 2$ and $\beta 3$ integrins to high affinity ligand binding states. This defect in inside-out signaling has been mapped to mutations in the protein Kindlin-3, which binds to specific domains within the cytoplasmic tail of β subunits, leading to separation of the cytoplasmic tails that is required as part of the unfolding of the integrin to form the ligand binding conformation (Abram and Lowell, ICB 87:440, 2009, Svensson et al Nat. Med, 15:306, 2009 Malinin et al Nat Med 15: 313, 2009). In these patients, their clinical presentation would suggest mainly $\beta 2$ (immune cell recruitment) and $\beta 3$ (platelet and osteoclast) functional defects. Indeed, Kindlin-3 expression is restricted to mainly hematopoietic cells. Mutations in ubiquitously expressed Kindlin-1 produce Kindler's Syndrome, which presents as a skin and mucosal blistering disease, due to defects in keratinocyte adhesion probably due to failure to activate $\beta 1$ integrins in these cells. As in the other integrin deficiencies, mice lacking either Kindlin-1 or Kindlin-3 model the respective human disease, though the *Kindlin-3* knockouts tend to have a less severe bleeding disorder (Moser et al Nat Med 15: 300, 2009). Mice lacking Kindlin-2, which is also widely expressed, die early in gestation due to implantation defects, potentially as a result of poor cell/cell adhesion (Montanez et al Genes Dev 15:1325, 2008).

Summary

Integrins are adhesive proteins that have evolved to allow cell-cell and cell-matrix communication that is indispensable for development and post-natal physiology. Despite their widespread expression, the genetic deletion of specific integrin family members in lower organisms as well as mammals leads to relatively distinct abnormalities. Many processes in which integrins participate, have in common a requirement for strong adhesion coincident with times of mechanical stress. As an example in *Drosophila*, the absence of specific integrins leads to detachment of muscle from the gut and body wall and separation of the two epithelial layers in the wing. In mice and humans, a deletion of either subunit of the laminin binding integrin, $\alpha 6\beta 4$ leads to severe skin blistering and defects in other epithelial layers due to problems in formation and stabilization of hemidesmosomes, which anchor epithelial cells to the basement membrane and are necessary for the skin to withstand frictional forces. However, integrins have also evolved to serve more subspecialized roles. These range from the establishment of a stem cell niche in *Drosophila* and mammals, to the regulation of pathogenic tumor vascularization, platelet adhesion and leukocyte transmigration in mammalian systems. On the other hand, some cells seem to function normally in the absence of all integrins, as revealed by the very surprising finding that deletion of all the major integrin types on dendritic cells of mice has no effect on the ability of these cells to migrate within the interstitium of the skin and enter into lymphatics. In addition to serving as transmembrane mechanical links, integrins in vertebrates synergize with other receptors including growth factor receptors. This leads to the activation of a large signaling network that affects cell proliferation and differentiation, cell shape and migration. The ability of integrins to directly bind and activate growth factors and interact with their receptors as is the case for VEGF, or modulate growth factor activity as is the case for TGF β , has major implications for adult homeostasis. In the latter case, the results from knockout mice may aid in the interpretation of observations in patients. That is, $\beta 6$ deletion in mice leads to a failure in activation of TGF β , which provides tonic suppression of inflammatory cells, and this may provide an explanation for the observed inflammation in patients with $\beta 6$ deficiency. These types of *in vivo* studies, in lower organisms, knock-out models as well as in human conditions together have provided important insights into how this major, primordial family of adhesion receptors have remained true to their name "integrins" as their diverse functions have in common the ability to integrate extracellular stimuli and transmit signals into cells that affect cell behavior.

Some of the challenges in the next decade are to understand how these receptors signal their diverse functions, how the same integrin can specialize to promote specific functions that are dependent on the environment (cell and tissue in which it exists) and how integrins collaborate with other integrins and receptors within a network to deliver cues responsible for cell processes that range from migration to differentiation. This type of knowledge will allow integrin-targeted therapeutic modalities that inhibit pathologic processes in which integrins have been heavily implicated including tumor angiogenesis, inflammation and thrombosis.

References

1. Hynes RO. Integrins: bidirectional, allosteric signaling machines. *Cell*. 2002; 110:673–687. [PubMed: 12297042]
2. Johnson MS, Lu N, Denessiouk K, Heino J, Gullberg D. Integrins during evolution: evolutionary trees and model organisms. *Biochim Biophys Acta*. 2009; 1788:779–789. [PubMed: 19161977]
3. Whittaker CA, Hynes RO. Distribution and evolution of von Willebrand/integrin A domains: widely dispersed domains with roles in cell adhesion and elsewhere. *Mol Biol Cell*. 2002; 13:3369–3387. [PubMed: 12388743]
4. Meighan CM, Schwarzbauer JE. Temporal and spatial regulation of integrins during development. *Curr Opin Cell Biol*. 2008; 20:520–524. [PubMed: 18603422]
5. Lauffenburger DA, Horwitz AF. Cell migration: a physically integrated molecular process. *Cell*. 1996; 84:359–369. [PubMed: 8608589]
6. Dustin ML. The cellular context of T cell signaling. *Immunity*. 2009; 30:482–492. [PubMed: 19371714]
7. Brown NH. Cell-cell adhesion via the ECM: integrin genetics in fly and worm. *Matrix Biol*. 2000; 19:191–201. [PubMed: 10936444]
8. Hynes RO, Zhao Q. The evolution of cell adhesion. *J Cell Biol*. 2000; 150:F89–96. [PubMed: 10908592]
9. Ellis SJ, Tanentzapf G. Integrin-mediated adhesion and stem-cell-niche interactions. *Cell Tissue Res*. 2009
10. Montell DJ. The genetics of cell migration in *Drosophila melanogaster* and *Caenorhabditis elegans* development. *Development*. 1999; 126:3035–3046. [PubMed: 10375496]
11. Grotewiel MS, Beck CD, Wu KH, Zhu XR, Davis RL. Integrin-mediated short-term memory in *Drosophila*. *Nature*. 1998; 391:455–460. [PubMed: 9461212]
12. Delon I, Brown NH. Integrins and the actin cytoskeleton. *Curr Opin Cell Biol*. 2007; 19:43–50. [PubMed: 17184985]
13. Sheppard D. In vivo functions of integrins: lessons from null mutations in mice. *Matrix Biol*. 2000; 19:203–209. [PubMed: 10936445]
14. Weis SM. Evaluating integrin function in models of angiogenesis and vascular permeability. *Methods Enzymol*. 2007; 426:505–528. [PubMed: 17697897]
15. McCarty JH. Integrin-mediated regulation of neurovascular development, physiology and disease. *Cell Adh Migr*. 2009; 3:211–215. [PubMed: 19372738]
16. Astrof S, Hynes RO. Fibronectins in vascular morphogenesis. *Angiogenesis*. 2009; 12:165–175. [PubMed: 19219555]
17. Yang JT, Rayburn H, Hynes RO. Embryonic mesodermal defects in alpha 5 integrin-deficient mice. *Development*. 1993; 119:1093–1105. [PubMed: 7508365]
18. Hynes RO. Cell-matrix adhesion in vascular development. *J Thromb Haemost*. 2007; 5(Suppl 1): 32–40. [PubMed: 17635706]
19. Stephens LE, Sutherland AE, Klimanskaya IV, Andrieux A, Meneses J, Pedersen RA, Damsky CH. Deletion of beta 1 integrins in mice results in inner cell mass failure and peri-implantation lethality. *Genes Dev*. 1995; 9:1883–1895. [PubMed: 7544312]

20. Carlson TR, Hu H, Braren R, Kim YH, Wang RA. Cell-autonomous requirement for beta1 integrin in endothelial cell adhesion, migration and survival during angiogenesis in mice. *Development*. 2008; 135:2193–2202. [PubMed: 18480158]
21. Brooks PC, Clark RA, Cheresh DA. Requirement of vascular integrin alpha v beta 3 for angiogenesis. *Science*. 1994; 264:569–571. [PubMed: 7512751]
22. Brooks PC, Montgomery AM, Rosenfeld M, Reisfeld RA, Hu T, Klier G, Cheresh DA. Integrin alpha v beta 3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. *Cell*. 1994; 79:1157–1164. [PubMed: 7528107]
23. De S, Razorenova O, McCabe NP, O'Toole T, Qin J, Byzova TV. VEGF-integrin interplay controls tumor growth and vascularization. *Proc Natl Acad Sci U S A*. 2005; 102:7589–7594. [PubMed: 15897451]
24. Soldi R, Mitola S, Strasly M, Defilippi P, Tarone G, Bussolino F. Role of alphavbeta3 integrin in the activation of vascular endothelial growth factor receptor-2. *EMBO J*. 1999; 18:882–892. [PubMed: 10022831]
25. Mahabeleshwar GH, Feng W, Phillips DR, Byzova TV. Integrin signaling is critical for pathological angiogenesis. *J Exp Med*. 2006; 203:2495–2507. [PubMed: 17030947]
26. Huang XZ, Wu JF, Ferrando R, Lee JH, Wang YL, Farese RV Jr, Sheppard D. Fatal bilateral chylothorax in mice lacking the integrin alpha9beta1. *Mol Cell Biol*. 2000; 20:5208–5215. [PubMed: 10866676]
27. Bazigou E, Xie S, Chen C, Weston A, Miura N, Sorokin L, Adams R, Muro AF, Sheppard D, Makinen T. Integrin-alpha9 is required for fibronectin matrix assembly during lymphatic valve morphogenesis. *Dev Cell*. 2009; 17:175–186. [PubMed: 19686679]
28. Vlahakis NE, Young BA, Atakilit A, Sheppard D. The lymphangiogenic vascular endothelial growth factors VEGF-C and -D are ligands for the integrin alpha9beta1. *J Biol Chem*. 2005; 280:4544–4552. [PubMed: 15590642]
29. Bader BL, Rayburn H, Crowley D, Hynes RO. Extensive vasculogenesis, angiogenesis, and organogenesis precede lethality in mice lacking all alpha v integrins. *Cell*. 1998; 95:507–519. [PubMed: 9827803]
30. McCarty JH, Monahan-Earley RA, Brown LF, Keller M, Gerhardt H, Rubin K, Shani M, Dvorak HF, Wolburg H, Bader BL, Dvorak AM, Hynes RO. Defective associations between blood vessels and brain parenchyma lead to cerebral hemorrhage in mice lacking alphav integrins. *Mol Cell Biol*. 2002; 22:7667–7677. [PubMed: 12370313]
31. McCarty JH, Lacy-Hulbert A, Charest A, Bronson RT, Crowley D, Housman D, Savill J, Roes J, Hynes RO. Selective ablation of alphav integrins in the central nervous system leads to cerebral hemorrhage, seizures, axonal degeneration and premature death. *Development*. 2005; 132:165–176. [PubMed: 15576410]
32. Zhu J, Motejlek K, Wang D, Zang K, Schmidt A, Reichardt LF. beta8 integrins are required for vascular morphogenesis in mouse embryos. *Development*. 2002; 129:2891–2903. [PubMed: 12050137]
33. Proctor JM, Zang K, Wang D, Wang R, Reichardt LF. Vascular development of the brain requires beta8 integrin expression in the neuroepithelium. *J Neurosci*. 2005; 25:9940–9948. [PubMed: 16251442]
34. Grazioli A, Alves CS, Konstantopoulos K, Yang JT. Defective blood vessel development and pericyte/pvSMC distribution in alpha 4 integrin-deficient mouse embryos. *Dev Biol*. 2006; 293:165–177. [PubMed: 16529735]
35. Yang JT, Rayburn H, Hynes RO. Cell adhesion events mediated by alpha 4 integrins are essential in placental and cardiac development. *Development*. 1995; 121:549–560. [PubMed: 7539359]
36. Kwee L, Baldwin HS, Shen HM, Stewart CL, Buck C, Buck CA, Labow MA. Defective development of the embryonic and extraembryonic circulatory systems in vascular cell adhesion molecule (VCAM-1) deficient mice. *Development*. 1995; 121:489–503. [PubMed: 7539357]
37. Walker J, Menko AS. Integrins in lens development and disease. *Exp Eye Res*. 2009; 88:216–225. [PubMed: 18671967]

38. Simirskii VN, Wang Y, Duncan MK. Conditional deletion of beta1-integrin from the developing lens leads to loss of the lens epithelial phenotype. *Dev Biol.* 2007; 306:658–668. [PubMed: 17493607]
39. De Arcangelis A, Mark M, Kreidberg J, Sorokin L, Georges-Labouesse E. Synergistic activities of alpha3 and alpha6 integrins are required during apical ectodermal ridge formation and organogenesis in the mouse. *Development.* 1999; 126:3957–3968. [PubMed: 10433923]
40. Milner R I, Campbell L. The integrin family of cell adhesion molecules has multiple functions within the CNS. *J Neurosci Res.* 2002; 69:286–291. [PubMed: 12125070]
41. Graus-Porta D, Blaess S, Senften M, Littlewood-Evans A, Damsky C, Huang Z, Orban P, Klein R, Schittny JC, Muller U. Beta1-class integrins regulate the development of laminae and folia in the cerebral and cerebellar cortex. *Neuron.* 2001; 31:367–379. [PubMed: 11516395]
42. Miyagoe-Suzuki Y, Nakagawa M, Takeda S. Merosin and congenital muscular dystrophy. *Microsc Res Tech.* 2000; 48:181–191. [PubMed: 10679965]
43. Benson DL, Schnapp LM, Shapiro L, Huntley GW. Making memories stick: cell-adhesion molecules in synaptic plasticity. *Trends Cell Biol.* 2000; 10:473–482. [PubMed: 11050419]
44. Denda S, Reichardt LF. Studies on integrins in the nervous system. *Methods Enzymol.* 2007; 426:203–221. [PubMed: 17697886]
45. Pietri T, Eder O, Breau MA, Topilko P, Blanche M, Brakebusch C, Fassler R, Thiery JP, Dufour S. Conditional beta1-integrin gene deletion in neural crest cells causes severe developmental alterations of the peripheral nervous system. *Development.* 2004; 131:3871–3883. [PubMed: 15253938]
46. Feltri ML, Graus Porta D, Previtali SC, Nodari A, Miglia vacca B, Cassetti A, Littlewood-Evans A, Reichardt LF, Messing A, Quattrini A, Mueller U, Wrabetz L. Conditional disruption of beta 1 integrin in Schwann cells impedes interactions with axons. *J Cell Biol.* 2002; 156:199–209. [PubMed: 11777940]
47. Nandrot EF, Kim Y, Brodie SE, Huang X, Sheppard D, Finnemann SC. Loss of synchronized retinal phagocytosis and age-related blindness in mice lacking alphavbeta5 integrin. *J Exp Med.* 2004; 200:1539–1545. [PubMed: 15596525]
48. Ley K, Laudanna C, Cybulsky MI, Nourshargh S. Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nat Rev Immunol.* 2007; 7:678–689. [PubMed: 17717539]
49. Berlin C, Bargatze RF, Campbell JJ, von Andrian UH, Szabo MC, Hasslen SR, Nelson RD, Berg EL, Erlandsen SL, Butcher EC. alpha 4 integrins mediate lymphocyte attachment and rolling under physiologic flow. *Cell.* 1995; 80:413–422. [PubMed: 7532110]
50. Abram CL, Lowell CA. The ins and outs of leukocyte integrin signaling. *Annu Rev Immunol.* 2009; 27:339–362. [PubMed: 19302044]
51. Scharffetter-Kochanek K, Lu H, Norman K, van Nood N, Munoz F, Grabbe S, McArthur M, Lorenzo I, Kaplan S, Ley K, Smith CW, Montgomery CA, Rich S, Beaudet AL. Spontaneous skin ulceration and defective T cell function in CD18 null mice. *J Exp Med.* 1998; 188:119–131. [PubMed: 9653089]
52. Kakkar AK, Lefer DJ. Leukocyte and endothelial adhesion molecule studies in knockout mice. *Curr Opin Pharmacol.* 2004; 4:154–158. [PubMed: 15063359]
53. Potocnik AJ, Brakebusch C, Fassler R. Fetal and adult hematopoietic stem cells require beta1 integrin function for colonizing fetal liver, spleen, and bone marrow. *Immunity.* 2000; 12:653–663. [PubMed: 10894165]
54. Sixt M, Bauer M, Lammermann T, Fassler R. Beta1 integrins: zip codes and signaling relay for blood cells. *Curr Opin Cell Biol.* 2006; 18:482–490. [PubMed: 16919433]
55. Hodivala-Dilke KM, McHugh KP, Tsakiris DA, Rayburn H, Crowley D, Ullman-Cullere M, Ross FP, Collier BS, Teitelbaum S, Hynes RO. Beta3-integrin-deficient mice are a model for Glanzmann thrombasthenia showing placental defects and reduced survival. *J Clin Invest.* 1999; 103:229–238. [PubMed: 9916135]
56. Pauls K, Schon M, Kubitza RC, Homey B, Wiesenborn A, Lehmann P, Ruzicka T, Parker CM, Schon MP. Role of integrin alphaE(CD103)beta7 for tissue-specific epidermal localization of CD8+ T lymphocytes. *J Invest Dermatol.* 2001; 117:569–575. [PubMed: 11564161]

57. Lammermann T, Bader BL, Monkley SJ, Worbs T, Wedlich-Soldner R, Hirsch K, Keller M, Forster R, Critchley DR, Fassler R, Sixt M. Rapid leukocyte migration by integrin-independent flowing and squeezing. *Nature*. 2008; 453:51–55. [PubMed: 18451854]
58. Pribila JT, Quale AC, Mueller KL, Shimizu Y. Integrins and T cell-mediated immunity. *Annu Rev Immunol*. 2004; 22:157–180. [PubMed: 15032577]
59. Berton G, Yan SR, Fumagalli L, Lowell CA. Neutrophil activation by adhesion: mechanisms and pathophysiological implications. *Int J Clin Lab Res*. 1996; 26:160–177. [PubMed: 8905448]
60. Hirahashi J, Mekala D, Van Ziffle J, Xiao L, Saffaripour S, Wagner DD, Shapiro SD, Lowell C, Mayadas TN. Mac-1 signaling via Src-family and Syk kinases results in elastase-dependent thrombohemorrhagic vasculopathy. *Immunity*. 2006; 25:271–283. [PubMed: 16872848]
61. Watt FM. Role of integrins in regulating epidermal adhesion, growth and differentiation. *EMBO J*. 2002; 21:3919–3926. [PubMed: 12145193]
62. Muller EJ, Williamson L, Kolly C, Suter MM. Outside-in signaling through integrins and cadherins: a central mechanism to control epidermal growth and differentiation? *J Invest Dermatol*. 2008; 128:501–516. [PubMed: 18268536]
63. Brakebusch C, Grose R, Quondamatteo F, Ramirez A, Jorcano JL, Pirro A, Svensson M, Herken R, Sasaki T, Timpl R, Werner S, Fassler R. Skin and hair follicle integrity is crucially dependent on beta 1 integrin expression on keratinocytes. *EMBO J*. 2000; 19:3990–4003. [PubMed: 10921880]
64. Raghavan S, Bauer C, Mundschauf G, Li Q, Fuchs E. Conditional ablation of beta1 integrin in skin. Severe defects in epidermal proliferation, basement membrane formation, and hair follicle invagination. *J Cell Biol*. 2000; 150:1149–1160. [PubMed: 10974002]
65. Chen J, Diacovo TG, Grenache DG, Santoro SA, Zutter MM. The alpha(2) integrin subunit-deficient mouse: a multifaceted phenotype including defects of branching morphogenesis and hemostasis. *Am J Pathol*. 2002; 161:337–344. [PubMed: 12107118]
66. DiPersio CM, Hodivala-Dilke KM, Jaenisch R, Kreidberg JA, Hynes RO. alpha3beta1 Integrin is required for normal development of the epidermal basement membrane. *J Cell Biol*. 1997; 137:729–742. [PubMed: 9151677]
67. Grose R, Hutter C, Bloch W, Thorey I, Watt FM, Fassler R, Brakebusch C, Werner S. A crucial role of beta 1 integrins for keratinocyte migration in vitro and during cutaneous wound repair. *Development*. 2002; 129:2303–2315. [PubMed: 11959837]
68. DiPersio CM, van der Neut R, Georges-Labouesse E, Kreidberg JA, Sonnenberg A, Hynes RO. alpha3beta1 and alpha6beta4 integrin receptors for laminin-5 are not essential for epidermal morphogenesis and homeostasis during skin development. *J Cell Sci*. 2000; 113(Pt 17):3051–3062. [PubMed: 10934043]
69. Kreidberg JA, Donovan MJ, Goldstein SL, Rennke H, Shepherd K, Jones RC, Jaenisch R. Alpha 3 beta 1 integrin has a crucial role in kidney and lung organogenesis. *Development*. 1996; 122:3537–3547. [PubMed: 8951069]
70. Aluwihare P, Munger JS. What the lung has taught us about latent TGF-beta activation. *Am J Respir Cell Mol Biol*. 2008; 39:499–502. [PubMed: 18927350]
71. Takabayashi K, Corr M, Hayashi T, Redecke V, Beck L, Guiney D, Sheppard D, Raz E. Induction of a homeostatic circuit in lung tissue by microbial compounds. *Immunity*. 2006; 24:475–487. [PubMed: 16618605]
72. Huang XZ, Wu JF, Cass D, Erle DJ, Corry D, Young SG, Farese RV Jr, Sheppard D. Inactivation of the integrin beta 6 subunit gene reveals a role of epithelial integrins in regulating inflammation in the lung and skin. *J Cell Biol*. 1996; 133:921–928. [PubMed: 8666675]
73. Morris DG, Huang X, Kaminski N, Wang Y, Shapiro SD, Dolganov G, Glick A, Sheppard D. Loss of integrin alpha(v)beta6-mediated TGF-beta activation causes Mmp12-dependent emphysema. *Nature*. 2003; 422:169–173. [PubMed: 12634787]
74. Pittet JF, Griffiths MJ, Geiser T, Kaminski N, Dalton SL, Huang X, Brown LA, Gotwals PJ, Kotliansky VE, Matthay MA, Sheppard D. TGF-beta is a critical mediator of acute lung injury. *J Clin Invest*. 2001; 107:1537–1544. [PubMed: 11413161]

75. Koth LL, Alex B, Hawgood S, Nead MA, Sheppard D, Erle DJ, Morris DG. Integrin beta6 mediates phospholipid and collectin homeostasis by activation of latent TGF-beta1. *Am J Respir Cell Mol Biol.* 2007; 37:651–659. [PubMed: 17641300]
76. Fjellbirkeland L, Cambier S, Broaddus VC, Hill A, Brunetta P, Dolganov G, Jablons D, Nishimura SL. Integrin alphavbeta8-mediated activation of transforming growth factor-beta inhibits human airway epithelial proliferation in intact bronchial tissue. *Am J Pathol.* 2003; 163:533–542. [PubMed: 12875973]
77. Su G, Hodnett M, Wu N, Atakilit A, Kosinski C, Godzich M, Huang XZ, Kim JK, Frank JA, Matthay MA, Sheppard D, Pittet JF. Integrin alphavbeta5 regulates lung vascular permeability and pulmonary endothelial barrier function. *Am J Respir Cell Mol Biol.* 2007; 36:377–386. [PubMed: 17079779]
78. Sparrow JC, Schock F. The initial steps of myofibril assembly: integrins pave the way. *Nat Rev Mol Cell Biol.* 2009; 10:293–298. [PubMed: 19190670]
79. Taverna D, Disatnik MH, Rayburn H, Bronson RT, Yang J, Rando TA, Hynes RO. Dystrophic muscle in mice chimeric for expression of alpha5 integrin. *J Cell Biol.* 1998; 143:849–859. [PubMed: 9813102]
80. Frisch SM, Screaton RA. Anoikis mechanisms. *Curr Opin Cell Biol.* 2001; 13:555–562. [PubMed: 11544023]
81. Campbell KP. Three muscular dystrophies: loss of cytoskeleton-extracellular matrix linkage. *Cell.* 1995; 80:675–679. [PubMed: 7889563]
82. Hayashi YK, Chou FL, Engvall E, Ogawa M, Matsuda C, Hirabayashi S, Yokochi K, Ziober BL, Kramer RH, Kaufman SJ, Ozawa E, Goto Y, Nonaka I, Tsukahara T, Wang JZ, Hoffman EP, Arahata K. Mutations in the integrin alpha7 gene cause congenital myopathy. *Nat Genet.* 1998; 19:94–97. [PubMed: 9590299]
83. Lopez MA, Mayer U, Hwang W, Taylor T, Hashmi MA, Jannapureddy SR, Boriek AM. Force transmission, compliance, and viscoelasticity are altered in the alpha7-integrin-null mouse diaphragm. *Am J Physiol Cell Physiol.* 2005; 288:C282–289. [PubMed: 15643051]
84. Mayer U, Saher G, Fassler R, Bornemann A, Echtermeyer F, von der Mark H, Miosge N, Poschl E, von der Mark K. Absence of integrin alpha 7 causes a novel form of muscular dystrophy. *Nat Genet.* 1997; 17:318–323. [PubMed: 9354797]
85. Nawrotzki R, Willem M, Miosge N, Brinkmeier H, Mayer U. Defective integrin switch and matrix composition at alpha 7-deficient myotendinous junctions precede the onset of muscular dystrophy in mice. *Hum Mol Genet.* 2003; 12:483–495. [PubMed: 12588796]
86. Rooney JE, Welser JV, Dechert MA, Flintoff-Dye NL, Kaufman SJ, Burkin DJ. Severe muscular dystrophy in mice that lack dystrophin and alpha7 integrin. *J Cell Sci.* 2006; 119:2185–2195. [PubMed: 16684813]
87. Welser JV, Rooney JE, Cohen NC, Gurpur PB, Singer CA, Evans RA, Haines BA, Burkin DJ. Myotendinous Junction Defects and Reduced Force Transmission in Mice that Lack {alpha}7 Integrin and Utrophin. *Am J Pathol.* 2009
88. Ross FP, Teitelbaum SL. alphavbeta3 and macrophage colony-stimulating factor: partners in osteoclast biology. *Immunol Rev.* 2005; 208:88–105. [PubMed: 16313343]
89. Horton MA, Massey HM, Rosenberg N, Nicholls B, Seligsohn U, Flanagan AM. Upregulation of osteoclast alpha2beta1 integrin compensates for lack of alphavbeta3 vitronectin receptor in Iraqi-Jewish-type Glanzmann thrombasthenia. *Br J Haematol.* 2003; 122:950–957. [PubMed: 12956766]
90. Mocsai A, Humphrey MB, Van Ziffle JA, Hu Y, Burghardt A, Spusta SC, Majumdar S, Lanier LL, Lowell CA, Nakamura MC. The immunomodulatory adapter proteins DAP12 and Fc receptor gamma-chain (FcRgamma) regulate development of functional osteoclasts through the Syk tyrosine kinase. *Proc Natl Acad Sci U S A.* 2004; 101:6158–6163. [PubMed: 15073337]
91. Faccio R, Teitelbaum SL, Fujikawa K, Chappel J, Zallone A, Tybulewicz VL, Ross FP, Swat W. Vav3 regulates osteoclast function and bone mass. *Nat Med.* 2005; 11:284–290. [PubMed: 15711558]

92. Bengtsson T, Aszodi A, Nicolae C, Hunziker EB, Lundgren-Akerlund E, Fassler R. Loss of alpha10beta1 integrin expression leads to moderate dysfunction of growth plate chondrocytes. *J Cell Sci.* 2005; 118:929–936. [PubMed: 15713743]
93. Popova SN, Barczyk M, Tiger CF, Beertsen W, Zigrino P, Aszodi A, Miosge N, Forsberg E, Gullberg D. Alpha11 beta1 integrin-dependent regulation of periodontal ligament function in the erupting mouse incisor. *Mol Cell Biol.* 2007; 27:4306–4316. [PubMed: 17420280]
94. Muller U, Wang D, Denda S, Meneses JJ, Pedersen RA, Reichardt LF. Integrin alpha8beta1 is critically important for epithelial-mesenchymal interactions during kidney morphogenesis. *Cell.* 1997; 88:603–613. [PubMed: 9054500]
95. Brandenberger R, Schmidt A, Linton J, Wang D, Backus C, Denda S, Muller U, Reichardt LF. Identification and characterization of a novel extracellular matrix protein nephronectin that is associated with integrin alpha8beta1 in the embryonic kidney. *J Cell Biol.* 2001; 154:447–458. [PubMed: 11470831]
96. Linton JM, Martin GR, Reichardt LF. The ECM protein nephronectin promotes kidney development via integrin alpha8beta1-mediated stimulation of Gdnf expression. *Development.* 2007; 134:2501–2509. [PubMed: 17537792]
97. Costantini F, Shakya R. GDNF/Ret signaling and the development of the kidney. *Bioessays.* 2006; 28:117–127. [PubMed: 16435290]
98. Liu Y, Chattopadhyay N, Qin S, Szekeres C, Vasylyeva T, Mahoney ZX, Taglienti M, Bates CM, Chapman HA, Miner JH, Kreidberg JA. Coordinate integrin and c-Met signaling regulate Wnt gene expression during epithelial morphogenesis. *Development.* 2009; 136:843–853. [PubMed: 19176588]
99. Zhang X, Mernaugh G, Yang DH, Gewin L, Srichai MB, Harris RC, Iturregui JM, Nelson RD, Kohan DE, Abrahamson D, Fassler R, Yurchenco P, Pozzi A, Zent R. beta1 integrin is necessary for ureteric bud branching morphogenesis and maintenance of collecting duct structural integrity. *Development.* 2009; 136:3357–3366. [PubMed: 19710172]
100. Kanasaki K, Kanda Y, Palmsten K, Tanjore H, Lee SB, Lebleu VS, Gattone VH Jr, Kalluri R. Integrin beta1-mediated matrix assembly and signaling are critical for the normal development and function of the kidney glomerulus. *Dev Biol.* 2008; 313:584–593. [PubMed: 18082680]
101. Kagami S, Kondo S. Beta1-integrins and glomerular injury. *J Med Invest.* 2004; 51:1–13. [PubMed: 15000250]
102. Cosgrove D, Rodgers K, Meehan D, Miller C, Bovard K, Gilroy A, Gardner H, Kotelianski V, Gotwals P, Amatucci A, Kalluri R. Integrin alpha1beta1 and transforming growth factor-beta1 play distinct roles in alport glomerular pathogenesis and serve as dual targets for metabolic therapy. *Am J Pathol.* 2000; 157:1649–1659. [PubMed: 11073824]
103. Hogg N, Bates PA. Genetic analysis of integrin function in man: LAD-1 and other syndromes. *Matrix Biol.* 2000; 19:211–222. [PubMed: 10936446]
104. Wilhelmsen K, Litjens SH, Sonnenberg A. Multiple functions of the integrin alpha6beta4 in epidermal homeostasis and tumorigenesis. *Mol Cell Biol.* 2006; 26:2877–2886. [PubMed: 16581764]
105. Kato A. The biologic and clinical spectrum of Glanzmann's thrombasthenia: implications of integrin alpha IIb beta 3 for its pathogenesis. *Crit Rev Oncol Hematol.* 1997; 26:1–23. [PubMed: 9246538]
106. Hynes RO, Hodivala-Dilke KM. Insights and questions arising from studies of a mouse model of Glanzmann thrombasthenia. *Thromb Haemost.* 1999; 82:481–485. [PubMed: 10605739]

Figure 1. The integrin family

18 α and 8 β subunits form 24 distinct, functional integrin pairs in humans and most likely in other vertebrates. Based on their evolutionary lineage, these can be grouped into subfamilies. The α subunits are grouped into those recognizing laminin (PS1 cluster) or the classical RGD sequence (PS2 cluster), and are clearly evolutionarily ancient receptors for ECM proteins. There is also a cluster of $\alpha4/\alpha9$ integrins, collagen receptors and leukocyte specific integrins. These subfamilies are further grouped into those containing the “inserted” I domain with structural similarity to the A domain found in von Willebrand factor. I domain containing integrins together with the $\alpha4/\alpha9$ cluster are restricted to chordates. These have evolved to bind counter receptors on vascular cells (e.g. ICAMs, VCAM), soluble molecules found in blood (e.g. fibrinogen, complement components) and pathogens (e.g. viruses and bacteria). While $\beta1$ and αv are ubiquitously expressed, $\beta2$ and $\beta7$ subunits are restricted to leukocytes. *Drosophila* evolved a small family of its own integrins, PS3-PS5 integrins for which there are no representatives in chordates.

Figure adapted from Johnson et al. 2009. Integrins during evolution: Evolutionary trees and model organisms. *Biochim. Biophys. Acta* 1788:779–89

Figure 2. Generalized organization of the integrin β subunits

The vertebrate β subunits fall into two major groups (A and B) while those present in invertebrates form a clearly distinct lineage. The Group A β subunits form most of the PS1, PS2 and I domain subgroups integrins with $\beta 2$ and $\beta 7$ subunits being restricted to hematopoietic cells. The Group B β subunits have a more restricted tissue distribution and associate with fewer α chains.

Figure from Johnson et al. 2009. Integrins during evolution: Evolutionary trees and model organisms. *Biochim. Biophys. Acta* 1788:779–89

Figure 3. Integrin signaling

In resting cells, integrins are held in a bent-inactive conformation (left most diagram). Following stimulation by any number of agonists (a generalized G-protein coupled receptor is depicted as a curved line) a number of signaling proteins are engaged which allow the assembly of the signaling complex on the cytoplasmic tails of the both the α and β subunits (left middle diagram). This induces an unfolding of the extracellular portion of the integrin to form a high affinity receptor. Shown are a number of proteins involved in these signaling proteins as well as some of the structural proteins that couple the integrin the actin cytoskeleton. This process is referred to as inside-out signaling events, since signals from inside the cell activate the integrin to a high affinity state. Following binding of the ligand to the integrin (shown in the right panels), additional signaling molecules are recruited to the tails of the integrins to lead to activation of downstream functional responses. Many of the molecules that are recruited include tyrosine kinases (Src-family kinases as an example, indicated as SFK) and other signaling adaptors that are utilized for a number of different types of signaling pathways. Ligand binding also induces clustering of integrins in the plasma membrane (which is not shown for simplicity), with all these signaling molecules attached to form a macromolecular structure known as the focal adhesion. This entire process is referred to as outside-in signaling since signals from ligand binding to the integrin receptor are communicated into the cell to lead to functional responses, such as actin polymerization and changes in gene transcription.

Figure adapted from Abram and Lowell 2009. The ins and outs of leukocyte integrin signaling. *Annu. Rev. Immunol.* 27:339–362

Figure 4. Integrin dependent leukocyte recruitment

The steps in leukocyte rolling, arrest, firm adhesion and diapedesis out of the vasculature and into the tissues are schematized. As shown, the initial steps of rolling and slow rolling are largely selectin mediated, however a number of integrins (such as $\alpha 4 \beta 1$) contribute to rolling events. Following cellular activation (which leads to inside-out signaling events and integrin affinity upregulation), leukocytes become firmly adherent to the endothelium and begin the process of crossing through (or around) the vascular endothelial cells. Integrin outside-in signaling events are important in mediating the actin cytoskeletal changes that are needed for cellular shape change during these steps. Direct interactions between the leukocyte and the basement membrane also guide the leukocyte out of the vasculature. Figure adapted from Ley et al 2007 Getting to the site of inflammation: the leukocyte adhesion cascade updated. *Nat. Rev. Immunol.* 7:678–689.

Table 1

The major ligands of human integrins

Integrins	Ligands	Primary sites of expression
<i>PS1 cluster – laminin receptors</i>		
$\alpha 3\beta 1$	Laminin, thrombospondin, uPAR	Skin, kidney, lung
$\alpha 6\beta 1$	Laminin, thrombospondin, ADAM, Cyr61	Macrophages, platelets
$\alpha 6\beta 4$	Laminins	Skin (hemi-desmosomes)
$\alpha 7\beta 1$	Laminins	Muscle
<i>PS2 cluster – RGD receptors</i>		
$\alpha IIb\beta 3$	Fibrinogen, thrombospondin, fibronectin, vitronectin, vWF, Cyr61, ICAM-4, CD40L	Platelets
$\alpha 5\beta 1$	Fibronectin, osteopontin, fibrillin, thrombospondin, ADAM, COMP	Blood vessels (embryonic)
$\alpha 8\beta 1$	Tenascin, fibronectin, osteopontin, vitronectin, latent TGF β , nephronectin	Kidney, inner ear
$\alpha v\beta 1$	Latent TGF β , fibronectin, osteopontin, vitronectin	Poorly defined
$\alpha v\beta 3$	Fibrinogen, vitronectin, fibronectin, vWF, thrombospondin, fibrillin, tenascin, PECAM-1, osteopontin, ADAM, Cyr61, MMP, uPAR, uPA, ICAM-4	Osteoclasts, vascular endothelium
$\alpha v\beta 5$	Osteopontin, vitronectin, latent TGF β	Eye, bone
$\alpha v\beta 6$	Latent TGF β , fibronectin, osteopontin, ADAM	Skin, lung
$\alpha v\beta 8$	Latent TGF β , vitronectin	Vascular endothelium
<i>$\alpha 4/\alpha 9$ cluster – chordate specific</i>		
$\alpha 4\beta 1$	Thrombospondin, MAdCAM, VCAM-1, ICAM-4, fibronectin	Heart (embryonic)
$\alpha 4\beta 7$	MAdCAM, VCAM-1, fibronectin	Gut homing T-cells (Peyers Patch)
$\alpha 9\beta 1$	Tenascin, VCAM-1, osteopontin, ADAM, VEGF-C, VEGF-D	Lymphatic endothelium
<i>I-domain containing integrins</i>		
<i>$\alpha 1, \alpha 2, \alpha 10, \alpha 11$ cluster – collagen receptors</i>		
$\alpha 1\beta 1$	Collagens, semaphorin 7A	Fibroblasts, mesenchymal tissues
$\alpha 2\beta 1$	Collagens, tenascin, E-cadherin	platelets, epithelium, fibroblasts, mesenchymal tissues,
$\alpha 10\beta 1$	Collagens	Cartilage, chondrocytes
$\alpha 11\beta 1$	Collagens	Peridontal ligaments
<i>$\alpha D, \alpha E, \alpha L, \alpha M, \alpha X$ cluster – leukocyte adhesion receptors</i>		
$\alpha D\beta 2$	ICAMs, VCAM-1, fibrinogen, plasminogen	Eosinophils
$\alpha E\beta 7$	E-cadherin	Skin/gut homing T and B- lymphocytes
$\alpha L\beta 2$	ICAMs	Leukocytes (all types)
$\alpha M\beta 2$	ICAMs, iC3b, fibrinogen, heparin, factor X	Myeloid leukocytes
$\alpha x\beta 2$	iC3b, fibrinogen, heparin, collagen, plasminogen, ICAM-4	Most myeloid leukocytes