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Regulatory mechanisms underlying T cell integrin receptor function

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Adhesion molecules allow lymphocytes to interact with and respond to the extracellular environment. Since these interactions must be essentially transient in nature, the function of lymphocyte adhesion molecules must be precisely regulated. Studies of integrin receptors vividly illustrate the various mechanisms by which the function of these adhesion molecules can be regulated. These include: (1) activation-dependent changes in functional activity; (2) changes in levels of expression due to differentiation events; (3) cell-specific differences in integrin binding; and (4) differential binding to distinct ligands by the same integrin. These mechanisms provide highly precise and specific modes of regulating lymphocyte interactions with a wide variety of potential counter-receptors and ligands.

Key words: T lymphocyte / integrin / adhesion / extracellular matrix / regulation

THE PROCESS OF ADHESION is of fundamental importance to the biology and development of multicellular organisms. For example, the development of complex multicellular organ systems cannot occur without physical contact of cells with other cells and components of the extracellular matrix (ECM). The importance of adhesion receptors has been particularly well illustrated in studies of the immune system, which, unlike other organ systems, is essentially composed of individual, free flowing cellular entities that must, at the appropriate time and place, interact with other cellular and extracellular components in order to respond to a foreign challenge. Such adhesive interactions result from the engagement of multiple cell surface receptors with various ligands or counter-receptors, many of which have been shown to transduce intracellular signals that subsequently impact on the functional responses mediated by the interacting cell(s).[1,2] The interaction of a T lymphocyte with an antigen-presenting cell and the adhesion of leukoctyes to vascular endothelial cells are two prominent immunological examples of cell-cell interactions where various adhesion molecules have been shown to play an absolutely essential role.[1,3]

The potential number of cell surface receptors that can mediate lymphocyte adhesion has increased in direct proportion to the interest among immunologists in cell adhesion. The focus of this review is on the integrins, a family of cell adhesion molecules that are utilized by many different cell types, including lymphocytes.[4] At the cell surface, an integrin receptor consists of two non-covalently associated transmembrane polypeptides, designated α and β. At present, 20 distinct integrin receptors have been defined, due to: (1) the existence of multiple α-chains and multiple β-chains; and (2) the ability of many α- and β-chains to associate with multiple partner polypeptides (Figure 1). Although most cell types express at least one integrin, an individual cell does not express all of the known integrins. Resting peripheral T cells express the β1 or VLA integrins $\alpha3\beta1$ (also VLA-3), $\alpha4\beta1$ (also VLA-4), $\alpha5\beta1$ (VLA-5), and $\alpha6\beta1$ (VLA-6), as well as the β2 integrin LFA-1 (also $\alpha L\beta2$).[5,6] A subpopulation of T cells also expresses the $\alpha4\beta7$ integrin, which has been postulated to mediate the specific migration of these T cells to Peyer's patches.[7] The counter-receptors for integrin molecules fall into three distinct categories: cell surface molecules such as ICAM-1 and VCAM-1, ECM components such as fibronectin (FN) and laminin, and bacterial or viral proteins, such as invasin (Table 1).[1,2,4,8] Most integrin receptors have more than one counter-receptor and most integrin counter-receptors are recognized by more than one integrin.

Recent progress in our understanding of the importance of integrins to lymphocyte function has arisen from three fronts. First, the identification of the various integrin ligands and counter-receptors continues to yield insights into the role of integrins in T cell recognition and migration. For example, the ability of β1 integrins on T cells to mediate

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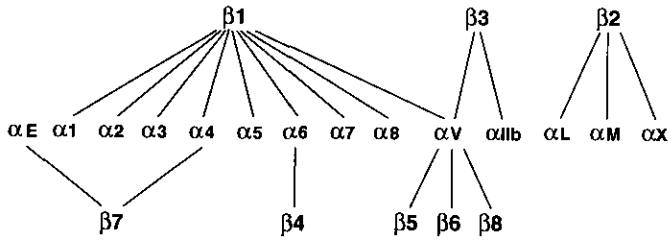

Figure 1. The integrin family of cell adhesion receptors. Each integrin receptor consists of an α-chain non-covalently associated with a β-chain at the cell surface. The various α-β pairings that have been described in various cell types are indicated by the lines between α- and β-chains.

adhesion to ECM components such as FN has led to a re-evaluation of the role of the ECM in T cell function.[2,5] Second, integrins expressed on various cell types have been shown to transduce signals upon engagement with a counter-receptor.[4] Although not the focus of this review, signalling by integrins is undoubtedly of critical importance to our understanding of integrin function. Third, since lymphocytes must continually alternate between adhesive and non-adhesive states, the analysis of lymphocyte integrins has been particularly important in our understanding of how lymphocyte adhesion is regulated.

This review will summarize our current understanding of the mechanisms that regulate the functional activity of integrin receptors. We discuss

Table 1. T cell integrins

Name		Expression pattern	Counter-receptor	ECM ligand	Other ligand
α1β1	VLA-1	*In vitro* activated T cells, specialized T cell subsets *in vivo*		Collagen*, laminin*	
α2β1	VLA-2	*In vitro* activated T cells, specialized T cell subsets *in vivo*		Collagen, laminin?*	Echovirus?*
α3β1	VLA-3	Low levels on resting T cells		Collagen, laminin?*	
α4β1	VLA-4	Resting peripheral T cells	VCAM-1, others?	Fibronectin, thrombo-spondin	Invasin†
α5β1	VLA-5	Resting peripheral T cells		Fibronectin, thrombo-spondin	
α6β1	VLA-6	Resting peripheral T cells		Laminin	
α4β7	LPAM-1, α4βp	Mouse lymphocytes, activated human T cells	Peyer's Patch HEV?, VCAM-1?	Fibronectin	
αEβ7	HML-1	Mucosal T cells			
αLβ2	LFA-1	Resting peripheral T cells	ICAM-1, ICAM-2, ICAM-3		
αMβ2	Mac-1	Subset of CD8+ T cells	ICAM-1	Fibrinogen	C3bi, LPS
αXβ2	p150/95	Some cytotoxic T cell clones	Endothelial ligand?	Fibrinogen	
αvβ3	VNR	Mouse T cell clones		Vitronectin, fibronectin, fibrinogen	

*These integrin receptor/ligand interactions have been established using cell types other than T cells.
†T cell binding to invasin is mediated predominantly through α4β1.

four general mechanistic themes: (1) qualitative changes in integrin functional activity induced by activation; (2) quantitative changes in integrin expression induced by differentiation; (3) cell-specific differences in integrin binding; and (4) differential binding to distinct counter-receptors by the same integrin. Although the first two themes have been the subject of numerous investigations, the latter two themes represent newly emerging principles of integrin receptor regulation. We have chosen to focus on studies of T lymphocyte integrin function, although these regulatory mechanisms are also likely to apply to other lymphoid and non-lymphoid cell types.

Qualitative changes in integrin functional activity by activation

Since integrins are essential for such fundamental T cell functions as interaction with antigen presenting cells, cytolytic effector function and migration and localization,[1] a deficiency in integrin-mediated adhesiveness would significantly reduce the effectiveness of an immune response. Likewise, constitutive, unregulated integrin adhesion would be equally detrimental to immune function. Therefore, T cells have developed multiple modes by which their adhesiveness is regulated. For integrins, one of the most important regulatory mechanisms is the rapid transition from a non-adhesive (low avidity) state to a transient adhesive (high avidity) state in response to an appropriate stimulus.

Extensive analysis of LFA-1-mediated adhesion of T cells to purified ICAM-1 by Dustin and Springer first demonstrated activation-dependent regulation of T cell integrin functional activity.[9] T cell adhesion to ICAM-1 was shown to be rapidly increased following acute T cell activation with the phorbol ester PMA, or by crosslinking the antigen-specific CD3/T cell receptor (CD3/TCR) complex. This mode of adhesion molecule regulation is novel for a variety of reasons. First, it is rapid. Increased LFA-1-mediated adhesion is detectable within 5 min of the delivery of the activation signal, and peaks by 10 min post activation. Second, depending on the activation signal, the transience of the adhesion can be equally rapid, with binding returning to pre-activation levels by 60 min. Third, the increase in adhesion occurs without an increase in the level of LFA-1 expression on the T cells. Thus, such activation-dependent regulation satisfies the requirements of rapid induction and reversibility needed for proper T cell function.

Activation-dependent regulation is not limited to LFA-1, but is a common function of all T cell integrins tested thus far. Resting human peripheral blood T cells express the $\beta1$ integrins $\alpha4\beta1$, $\alpha5\beta1$, and $\alpha6\beta1$, but only bind strongly to FN, VCAM-1, and laminin after activation with a variety of stimuli[5,10,11] (see below). As with LFA-1, this increase in $\beta1$-mediated adhesion occurs without an increase in the level of $\beta1$ integrin expression on the T cell surface.[5] In addition, although not expressed on resting human peripheral T cells, $\alpha2\beta1$ also demonstrates activation-dependent binding to collagen on both human and murine T cell lines.[12]

Many different modes of T cell activation can upregulate integrin function. In addition to pharmacologic agents such as PMA and the Ca^{2+} ionophore A23187, various cell surface receptors on the T cell, when activated by MAb-mediated crosslinking or natural ligand, can upregulate integrin activity. These include the CD3/TCR, CD2, CD7, CD28, and CD31, all of which have been shown to upregulate both $\beta1$- and $\beta2$-mediated T cell adhesion to various ligands and counter-receptors.[5,9,11-14] In addition, MIP-1β, a cytokine belonging to the intercrine/chemokine family that includes IL-8 and RANTES, has been shown to upregulate adhesion of $CD8^+$ T cells to VCAM-1 and FN.[15]

Why is there a need for so many different 'triggers' of integrin activity on the T cell surface? We speculate that different regulatory molecules upregulate integrin activity depending on the specific adhesive interactions occurring in a given microenvironment. Two such contexts are T cell interactions with antigen presenting cells and T cell adhesion to vascular endothelium. With regard to antigen recognition, the ability of CD3/TCR-mediated signalling to upregulate both $\beta1$ and $\beta2$ integrin-mediated adhesion suggests that T cell encounter with a specific foreign antigen serves to promote strong adhesion to the relevant antigen presenting cell as well as immobilize the T cell by adhesion to the surrounding ECM. Other receptors that regulate integrin activity, such as CD2 and CD28, have also been shown to transduce signals that facilitate CD3/TCR-mediated T cell activation.[16] It is likely that the engagement of multiple integrin-activating molecules during antigen presentation could alter integrin adhesiveness either quantitatively, by summation of similar intracellular signals, or qualitatively, by integration of dissimilar signals. There is some evidence for qualitative signalling differences, based on differential kinetics of adhesion

after CD3/TCR- or CD2-mediated activation.[13] Thus, for example, the role of CD2 in regulating integrin activity may be to prolong integrin-mediated adhesion that is initiated by the CD3/TCR. Other receptors, such as CD28 and potentially CD7, may serve similar functions when they are activated by ligand engagement during the process of antigen recognition. In fact, we hypothesize that multiple integrin-activating molecules are sequentially engaged during antigen recognition, insuring a sustained and functionally productive interaction between the activated T cell and the extracellular environment.

Activation-dependent upregulation of integrin activity has also been proposed to be a key step in the successful adhesion of a lymphocyte in the blood stream to vascular endothelial cells.[3,17] This 'adhesion cascade' has been proposed to involve: (1) initial contact of leukocytes mediated by members of the selectin family of adhesion molecules, causing the leukocytes to roll on the endothelial surface; and (2) subsequent delivery of an activation signal resulting in strong integrin-mediated attachment.[17] Although selectin-mediated adhesion events and cytokines such as IL-8 have been proposed to provide such triggering signals for neutrophils, the nature of the signals that upregulate lymphocyte integrin activity during interactions with endothelium are poorly defined. Although direct evidence is lacking, both CD31 and MIP-1β have been proposed to function as lymphocyte integrin triggering structures on endothelium.[14,15] The preferential expression of CD31 on certain T cell subsets, and the ability of MIP-1β to preferentially upregulate integrin function on $CD8^+$ T cells, suggests that these molecules may serve to mediate the subset-specific localization of T cells *in vivo*.[14,15] Thus, we would predict that there exist other triggering structures on endothelium that specifically upregulate integrin activity on other T cell subpopulations.

Insights into mechanisms of activation-dependent integrin function

Despite the importance of regulation of integrin molecules, the mechanism by which activation rapidly induces upregulation of integrin function still remains undefined. The ability of factors other than surface receptor ligation and cytokines to regulate integrin activity has provided some clues to potential regulatory mechanisms. Perhaps the most potent non-receptor activators of β1 and β2 integrins are phorbol esters, which directly stimulate the serine/threonine kinase protein kinase C (PKC), thereby implicating protein phosphorylation in integrin activation. Furthermore, specific PKC inhibitors not only inhibit PMA-induced integrin activation, but also significantly inhibit CD3-mediated upregulation of LFA-1 and β1 integrin activity.[9,13]

Although PKC-mediated protein phosphorylation is an important intracellular signal in the regulation of integrin function, it is clear that other intracellular signals are involved. First, pretreatment of T cells with cAMP analogs partially inhibits receptor-mediated integrin activation, but has no inhibitory effect on PMA-induced integrin activation,[9,11] implying that receptor ligation on T cells produces an integrin-activating signal distinct from PKC that is inhibited by cAMP. Second, integrin activation of the T cell line H9 can be induced by ligation of CD3, but not by PMA (J.L. Mobley, manuscript in preparation). Third, the calcium ionophore A23187 can induce integrin activation in peripheral T cells that is insensitive to specific PKC inhibitors,[11] whereas A23187 has no effect on integrin function in the Jurkat T cell line (J.L. Mobley, manuscript in preparation), suggesting the importance of calcium, perhaps as a co-factor for a T cell kinase not found or inactive in Jurkat T cells.

Recent reports of the regulation of B cell integrin function suggest that PKC mediates the rapid, transient integrin activation that is inhibitible by cAMP, but that this is followed 5-8 h later by a more prolonged cAMP-dependent, PKC-independent integrin activation.[18] Still other reports suggest that a protein phosphatase is responsible for the induction of B cell integrin activation,[19] or for the deactivation of T cell integrins leading to a reversal of adhesive function induced by PMA.[20] These results clearly implicate multiple key early second messengers in regulation of integrin functional activity, although an integrated mechanistic model is still not available.

The identification of PKC as an intracellular signal involved in the regulation of integrin function led to the hypothesis that integrin activation might be a consequence of integrin phosphorylation, perhaps resulting in a conformational change in the integrin ligand-binding domain. In fact, phosphorylation of serine and threonine residues on the cytoplasmic domain of the LFA-1 β2-chain does occur after PMA activation of T cells, although CD3-mediated activation fails to result in such phosphorylation.[21] The cytoplasmic domain of the β-chain is undoubtedly

involved in integrin activation, as evidenced by loss of PMA-induced integrin activation in transfectants expressing β2 integrins lacking the β2 cytoplasmic domain.[22] However, selective mutation or deletion of serines in the LFA-1 β2 cytoplasmic domain that are phosphorylated following PMA treatment does not alter the ability of PMA to upregulate LFA-1, highly suggestive that direct phosphorylation of LFA-1 is not a major mechanism of regulating integrin function.[23]

An alternative regulatory mechanism may be linkage of the integrin receptor via its cytoplasmic domains to cytoskeletal components. Several lines of evidence suggest that cytoskeletal association is required for activation-dependent integrin function. First, integrin-mediated adhesion in general and activation-dependent upregulation of integrin activity is inhibited by treatment of T cells with cytochalasin B, which disrupts actin microfilaments.[11,24] Second, T cell activation results in the capping of β1 and β2 integrins.[21] Third, the cytoskeletal proteins α-actinin and vinculin are associated with LFA-1 in T cells stimulated with PMA or anti-CD3 MAbs, but not in unstimulated T cells.[21] Another cytoskeleton element, talin, co-clusters with LFA-1 upon T cell-antigen-presenting cell interaction and is a substrate for phosphorylation by PKC.[25] Thus, talin may represent a common functional link between the requirement for both PKC activity and cytoskeletal integrity in integrin activation. Activation-dependent association of integrins with the cytoskeleton would provide a mechanism for the directed redistribution of integrins to a localized focus of ligand concentration, as would be found in the area of cell-cell contact between a T cell and an APC, resulting in a rapid increase in the avidity of interaction between the two cells. Although this model does not propose a role for the cytoskeleton in altering the affinity of an individual integrin for a ligand or counter-receptor, changes in cytoskeletal linkage caused by phosphorylation or other intracellular signalling events have also been proposed to alter receptor affinity by changing the conformation of the extracellular ligand-binding domain.[9]

Other studies also clearly suggest that upregulation of integrin functional activity may involve conformational changes that result in increased receptor affinity. Changes in the concentration of specific cations, particularly Mg^{2+} and Mn^{2+}, can dramatically increase integrin-mediated adhesion to various ligands and counter-receptors,[26-30] and may actually change the ligand specificity altogether[31] (see below). Changes in divalent cation concentrations have also been shown to induce novel epitopes on β2 integrins that are detectable by MAbs.[26] Localized changes in divalent cation concentrations (as may be found at sites of tissue injury) have been proposed as a mechanism by which cations can directly modulate integrin activity and consequently alter cell migration.[30] It has also been proposed that an activation stimulus results in a conformational alteration in the integrin ligand-binding region associated with the displacement of one divalent cation with another that is more permissive for integrin ligand binding.[26]

In addition to cation-induced integrin activation, some integrin-specific MAbs can directly upregulate integrin binding.[32-35] The mode of action of these MAbs appears to be somewhat distinct, based on differential requirements for an intact cytoskeleton, metabolic energy or a particular cation. Nevertheless, these studies imply that these MAbs induce changes in integrin conformation that result in increased functional activity. These changes may be similar in nature to those induced by divalent cation modifications.

Integrin function may also be influenced by the lipid content of the cell membrane. Integrin modulating factor-1 (IMF-1) is a fatty acid or isoprenoid factor purified from the membranes of activated neutrophils that induces a transient activation of the β2 integrin Mac-1 on neutrophils.[36] As of yet, however, there are no reports of similar fatty acid integrin regulators in lymphocytes. The membrane gangliosides GD2 and GD3 have also been implicated in the regulation of integrin function because: (1) antibodies specific for GD2 and GD3 inhibit the binding of melanoma cells to collagen, vitronectin, laminin, and FN; and (2) these gangliosides copurify with the αvβ3 integrin on affinity columns in the presence of Ca^{2+}.[37] Lipids and glycolipids could have multiple effects on integrin function. First, lipids could change the conformation of the integrin ligand-binding region by direct association in the membrane, as discussed above for divalent cations and MAbs. Second, the surrounding lipid milieu could alter integrin mobility in the membrane bilayer, permitting the formation of integrin microclusters focused to discrete areas of high-avidity binding. Third, the sialic acid component of gangliosides has been proposed to bind and sequester divalent cations that alter the conformation of the integrin ligand-binding domain.[37]

Thus, the studies to date support two potential mechanisms for activation-dependent upregulation of integrin activity: (1) induced integrin microclustering in the cell membrane, resulting in increased avidity of binding; and (2) induced and transient changes in the conformation of the integrin extracellular domain, resulting in increased receptor affinity. There are several important general points to mention. First, these mechanisms need not be mutually exclusive. Second, it is currently not known if all of the various modes by which T cell integrins can be upregulated utilize common or distinct mechanistic pathways. Third, insights into regulation of integrin activity on lymphocytes have been preceded by extensive analysis of similar activation-dependent changes of the platelet integrin gpIIb/IIIa.[38]

Quantitative changes in integrin expression induced by differentiation

In addition to transient, qualitative changes in integrin receptor activity, stable change in levels of integrin expression on distinct T cell subsets is another major regulatory mechanism. Although $\alpha1\beta1$ and $\alpha2\beta1$ are not expressed on resting peripheral T cells, these integrins are expressed on the surface of chronically activated T cells *in vitro* and on specialized T cell subsets *in vivo*.[39,40] Since $\alpha1\beta1$ and $\alpha2\beta1$ have been shown to bind collagen, expression of these integrins consequently expands the range of ECM ligands to which these cells can bind. In fact, collagen binding via these integrins has been proposed as a potential mechanism by which $\alpha1\beta1$-positive T cells in lung epithelium are prevented from escaping into the periphery.[40]

Resting human T cells also show differential integrin expression. In fact, MAbs specific for the $\beta1$-chain have been used as markers for two distinct T cell subsets.[41] MAbs specific for the CD45 isoforms CD45RA and CD45RO are the most widely used discriminators for these subsets, which have been functionally designated as 'naïve' ($CD45RA^+$) and 'memory' T cells ($CD45RO^+$) (for a recent review see ref 42). Since response to recall antigen is found in the CD45 RO^+ memory subpopulation, it has been proposed that naïve cells represent T cells that have yet to encounter antigen in the periphery, but that upon antigen exposure differentiate into resting memory T cells.[42] Although there is conflicting evidence regarding the functional interrelationship between $CD45RA^+$ and $CD45RO^+$ T cells, a clear phenotypic distinction between these T cell subsets is the differential expression of integrins and other adhesion molecules.

Flow cytometric analysis has shown that although all $CD4^+$ T cells express LFA-1, $\alpha4\beta1$, $\alpha5\beta1$, and $\alpha6\beta1$, there is a 2- to 4-fold higher level of expression of each of these integrins on $CD4^+CD45RO^+$ T cells compared to $CD4^+$ $CD45RA^+$ T cells.[5,6] Consequently, $CD4^+$ $CD45RO^+$ T cells show greater levels of adhesion to purified integrin ligands and counter-receptors as well as intact cells that express integrin ligands, such as endothelium.[5,10] The differences in integrin expression between naïve and memory T cells may be critical to (1) differential functional responses of these two subsets to activation, and (2) differential migratory patterns *in vivo*.[43] Recent studies also suggest further complexities in integrin expression in peripheral T cells, particularly in the CD45 RO^+ subpopulation.[44,45] For example, Horgan *et al* observed that while expression of $\alpha3$, $\alpha5$ and $\alpha6$ in $CD4^+CD45RO^+$ T cells parallels the expression of $\beta1$, $\alpha4$ expression was strikingly discordant, and could subdivide T cells into two smaller subsets defined by low and high $\alpha4$ expression.[45] Thus, it has been proposed that memory T cell subsets support a 'molecular imprinting' model by which a T cell, upon differentiation from the naïve to the memory cell-type, acquires a specific migratory capacity dictated by the differential expression of integrins and other molecules yet to be defined.

In addition to differential expression of the integrins themselves, molecules implicated in upregulating integrin functional activity are also differentially expressed on T cells.[11,14] While CD3 is uniformly expressed on both naïve and memory T cells, there is greater expression of CD2 and CD28 on memory T cells (ref 6; Y. Shimizu, unpublished). Conversely, CD7 shows 2- to 3-fold greater levels of expression on $CD4^+$ naïve T cells.[11] While crosslinking of CD2 or CD28 preferentially increases memory T cell adhesion to FN and ICAM-1, CD7 crosslinking results in comparable levels of adhesion of both naïve and memory T cells.[11] CD31 shows even more complexity in expression. Within $CD4^+$ T cells, only a subset of $CD4^+CD45RA^+$ cells expresses appreciable levels of CD31. $CD8^+$ T cells show a different pattern of CD31 expression, with uniform expression on $CD8^+CD45RA^+$ T cells and bimodal expression on $CD8^+CD45RO^+$ T cells.[14] These differences in CD31 expression correlate with differences in the ability of various T cell subpopulations to bind to FN, VCAM-1 and ICAM-1 after

upregulation of integrin activity by CD31 MAbs. Thus, integrin function on peripheral T cells can be regulated not only by differential expression of the integrin receptors themselves, but also by the molecules that upregulate their activity. The expression pattern of such molecules on any given T cell is likely to dictate the specific pattern of migration to anatomic sites *in vivo*, since these molecules are thought to participate in sequential adhesion and activation events (i.e. 'adhesion cascades') when encountering an endothelial cell.[3,17] Similar types of adhesion cascades may also occur when a T cell encounters an antigen presenting cell and differential expression of integrins and their regulatory molecules may also be relevant to the differential functional responses of naïve and memory T cells.[42]

Cell-specific differences in integrin binding

Integrin ligands and counter-receptors have been identified by analyzing a wide variety of cell types including fibroblasts, endothelial cells, leukocytes, and platelets. It has been assumed that the ligand specificity of a particular integrin was the same irrespective of the cell type on which it was displayed. However, several recent studies have challenged that assumption. For example, activated T cells expressing equivalent amounts of $\alpha1\beta1$, $\alpha2\beta1$, and $\alpha3\beta1$ utilize only $\alpha2\beta1$ to bind to collagen,[46] even though all three of these integrins had previously been identified as a collagen-binding integrin when expressed on non-lymphoid cells.[4] Other evidence of cell type-specific integrin function involving $\alpha2\beta1$ has also been reported: $\alpha2\beta1$ expressed on a melanoma cell line[47] and on endothelial cells[48] binds to both laminin and collagen, whereas $\alpha2\beta1$ demonstrates no laminin-binding activity when expressed on the surface of platelets or fibroblasts.[47,48]

Such seemingly inconsistent integrin binding activity is not limited to $\alpha2\beta1$ or to ECM integrin ligands. Biochemical studies originally demonstrated that mammalian cell binding to invasin could be mediated by $\alpha3\beta1$, $\alpha4\beta1$, $\alpha5\beta1$, and $\alpha6\beta1$.[8] While resting T lymphocytes express all of these integrins, only $\alpha4\beta1$ appears to be involved in T cell adhesion to invasin.[49] In addition, an $\alpha5$-specific MAb that blocks the binding of the K562 erythroleukemia cell line to invasin[8] fails to block T cell binding, suggesting cell-specific differences in the ability of $\alpha5\beta1$ to bind to this novel bacterial counter-receptor.[49]

As of yet, there is no known mechanism for cell type-specific integrin function, but several hypotheses have been proposed. First, integrins expressed on different cells may be structurally different due to alternative mRNA splicing or differential glycosylation. Splice variants have been reported for several integrins (reviewed in ref 4), but not in the context of cell type-specific ligand binding. Extensive epitope analysis, molecular weight analysis, and N-terminal sequencing have failed to detect differences in $\alpha2\beta1$ in cells that show differential $\alpha2\beta1$-mediated adhesion to collagen and laminin.[47,48] However, in a metastatic murine melanoma line, mutant-specific glycosylation differences are reported to correlate with differential integrin ligand-binding activity and metastatic potential.[50]

Second, there may be cell type-specific accessory molecules that modify integrin-mediated binding. Gangliosides have been proposed as a candidate for this function due to their association with certain integrins (see above). In another receptor system, non-receptor accessory proteins associated with receptors for IL-3, IL-5, Il-6, and GM-CSF modulate ligand-binding affinities and confer cell type-specificity for cytokine binding (reviewed in ref 51).

Third, mechanisms that may play a role in the activation-induced regulation of integrin functional activity (as described above) may also be utilized in a cell-type-specific fashion. For example, cell-specific differences in membrane lipid content, constitutive levels of phosphorylation, cytoskeletal association and/or localized concentrations of divalent cations might serve to create cell type-specific differences in integrin conformation that result in differential ligand binding.[27,31]

Thus, cell type-specific ligand binding is likely to represent an important additional means of regulating integrin function because: (1) it expands the versatility of a finite number of integrin molecules; and (2) it provides a mechanism whereby distinct cell types can receive potentially novel signals from the same set of integrin ligands and counter-receptors.

Differential binding to distinct counter-receptors by the same integrin

A characteristic of T cell integrins, and integrins in general, is that they are capable of binding to multiple, distinct ligands and counter-receptors (Table 1). For example, $\alpha4\beta1$ has two ECM ligands (FN and thrombospondin), a mammalian cell surface

counter-receptor (VCAM-1) and a bacterial counter-receptor (invasin). Despite insights into specific peptide sequences that seem to be important in integrin binding,[4] it is still not known how an individual integrin, such as $\alpha4\beta1$, can discriminate between multiple ligands. Furthermore, there is emerging evidence that there may be differences in the regulation of binding by one integrin to its multiple different ligands and counter-receptors. For example, while strong $\alpha4\beta1$-mediated adhesion to VCAM-1 and FN requires T cell activation, strong binding to invasin can occur in the absence of activation.[49] There are also striking differences in divalent cation utilization in $\alpha4\beta1$-mediated cell adhesion to FN and VCAM-1,[28] suggesting differences in regulation of binding to these ligands. Thus, in addition to differential expression *in vivo* of various ligands, such as the striking differences in expression of the three LFA-1 counter-receptors ICAM-1, ICAM-2, and ICAM-3,[52] differential binding of an integrin to distinct counter-receptors is also likely to influence integrin-mediated T cell adhesion.

Signalling by integrins

Evidence from various fields including immunology has converged to show that integrins signal intracellularly upon binding a ligand or counter-receptor (reviewed in refs 4,53). This evidence includes: (1) enhancement of CD3- and superantigen-mediated T cell proliferation by various integrin ligands and counter-receptors, including ICAM-1, ICAM-2, VCAM-1, FN, laminin, collagen, and invasin (reviewed in refs 2,4,16); (2) increases in intracellular pH in endothelial cells, fibroblasts and lymphocytes after binding to ECM ligands or integrin-specific MAbs;[54] and (3) increases in tyrosine phosphorylation after cell activation, or more significantly, after MAb crosslinking of integrins or cell adhesion to ECM components.[55-58] In fibroblasts, KB carcinoma cells, and platelets, one of the proteins phosphorylated upon integrin ligation has been identified as $pp125^{FAK}$,[55,57-59] a tyrosine kinase and a putative substrate of the membrane associated tyrosine kinase $p60^{src}$. The phosphorylation of $pp125^{FAK}$, as well as cytoskeletal proteins such as paxillin, upon integrin binding and the co-localization of $pp125^{FAK}$ and integrins to focal adhesions suggests a model whereby integrins transduce signals via a phosphorylation cascade that may result in cytoskeletal rearrangements.[57]

Although the expression of $pp125^{FAK}$ in lymphoid cells has not been reported, induced tyrosine phosphorylation by $\alpha4\beta1$ engagement[56] suggests that similar signalling events also occur with lymphocyte integrins. Integrins may be similar in nature to other well-characterized signalling complexes expressed in lymphocytes, such as CD4, CD8, and the CD3/TCR, that transduce signals through associated intracellular tyrosine kinases. Thus, a complete understanding of integrin function in T cells will require further insights into intracellular signalling both to and from T cell integrins.

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