Review

The Biological Functions of β3 Integrins

(β3 integrins / αIIbβ3 integrin / αvβ3 integrin / biological functions)

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Abstract. Integrins comprise a large family of αβ heterodimeric cell-surface receptors that are found in many animal species. They are expressed on a wide variety of cells. There are two members in the β3 integrin family: αIIbβ3 and αvβ3. This class of adhesion receptors mediates cell-cell and cell-extracellular matrix interactions. Dysregulation of the β3 integrins is involved in the pathogenesis of many diseases (including cancer) and in transplant rejection. Integrins also play a key role in many virus infectious cycles. In this paper the biological functions of the β3 family are reviewed, with particular interest in its role in cancer progression and metastasis.

Integrins comprise a large family of αβ heterodimeric cell-surface receptors that are found in many animal species, ranging from sponges to mammals (Giancotti and Ruoslahti, 1999; Arnaout et al., 2002). They are expressed on a wide variety of cells, with most cells expressing several different integrins. There are only two members in the β3 integrin family: αIIbβ3 and αvβ3. This class of adhesion receptors mediates cell-cell and cell-extracellular matrix (ECM) interactions. Such interactions are important for the maintenance of tissue integrity, the promotion of cellular migration, the regulation of gene expression, and cell survival, adhesion and differentiation. They also have important functions in the development of a tissue, angiogenesis, wound healing, and thrombosis (Schwartz et al., 1995; Giancotti and Ruoslahti, 1999; Adair and Yeager, 2002; Vinogradova et al., 2002). Dysregulation of the β3 integrins is involved in the pathogenesis of many diseases (e.g. autoimmune diseases, cardiovascular disorders, and osteoporosis) and in transplant rejection. Integrins are able to mediate adhesive events during various cancer stages, such as malignant transformation, and tumour growth, progression, and metastasis. In particular, overexpression of integrin αvβ3 has been demonstrated in various tumours (Miziejewski, 1999; Li et al., 2001; Hosotani et al., 2002). It is known that some viruses may very often use integrins (from the β3 family) to attach to host cells (HIV – human immunodeficiency virus, HPEV-1 – human parechovirus 1, hantavirus, adenovirus) (Gavrilovskaya et al., 1999; Triantafilou et al., 2001; Lafrenie et al., 2002; Ling et al., 2002).

β3 integrin family

There are two members in the β3 integrin family: αIIbβ3 and αvβ3. The 40% sequence homology in the α subunits (αIIb and αv) of both β3 integrins suggests that they share the same basic structural elements (Mitchell et al., 2003).

αIIbβ3 integrin is a receptor expressed mainly on the surface of platelets and their precursors – megakaryocytes. It was also demonstrated that αIIbβ3 occurs on the surface of human blood monocytes, granulocytes, and large granular lymphocytes (Burns et al., 1986). This integrin plays a key role in platelet aggregation and thrombus formation. The main platelet receptor is inactive in resting cells, but after exposure to an agonist (such as ADP or thrombin) and/or platelet activator it changes to the active, extended form that can bind fibrinogen and other ligands (Basani et al., 2000; Shimaoka and Springer, 2003). Additionally, αIIbβ3

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Abbreviations: βTD – β-tail domain, CAV-9 – coxsackie virus A9, ECM – extracellular matrix, EGF – epidermal growth factor, FAK – focal adhesion kinase, FMDV – foot and mouth disease virus, GFR – growth factor receptor, GMK – green monkey kidney, HIV-1 – human immunodeficiency virus 1, HPEV-1 – human parechovirus 1, ILK – integrin-linked kinase, MAPK – mitogen-activated protein kinase, MIDAS – metal ion-dependent adhesion site, MMP2 – matrix metalloproteinase 2, PDGF – platelet-derived growth factor, PSI – plexins, semaphorins, integrins, TGF β1 – tumour growth factor β1, VEGF – vascular endothelial growth factor.

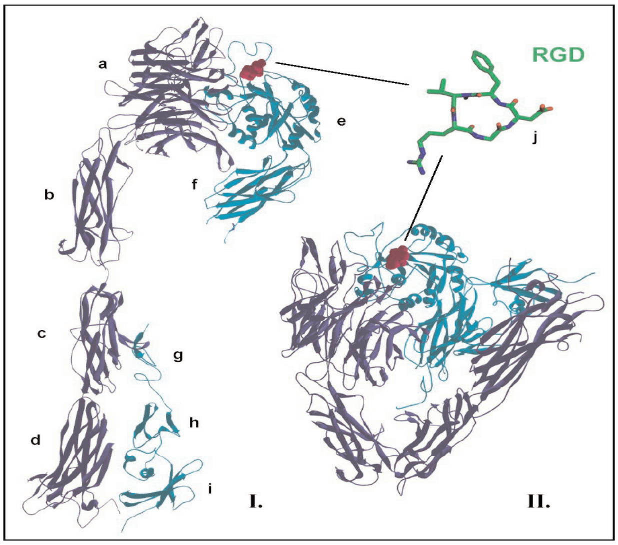

Fig. 1. Schematic representation of the extracellular portion of αvβ3 integrin in complex with an RGD ligand. (**I**) Simple model of the open conformation of the extended high-affinity "active" integrin based on Xiong et al. (2001); **a.** β propeller. **b.** Thigh domain. **c.** Calf-1 domain. **d.** Calf-2 domain. **e.** βA domain. **f.** Hybrid domain. **g.** EGF-3 domain. **h.** EGF-4 domain. **i.** βTD domain. **j.** Cyclic peptide Arg-Gly-Asp-Phe-Mva mimicking the RGD motif. PSI, EGF-1 and EGF-2 are not visible. (**II**) Low affinity "inactive" conformation as in crystal structure (PDB code: 1l5g). Chain αv in dark blue, chain β3 in cyan.
(Figure was obtained by using a Swiss-PdbViewer).

integrins are also mobilized to the platelet surface from an α-granule storage pool (Bennett, 2001). αIIbβ3 integrin dysfunctions are important in the pathogenesis of thrombotic cardiovascular diseases. This subgroup of integrins can bind to a large number of extracellular matrix proteins and numerous microorganisms can utilize integrins to gain entry into cells (Plow et al., 2000).

αvβ3 integrin is expressed on the surface of endothelial cells, smooth muscle cells, monocytes, and platelets. It is also known that αvβ3 integrin-mediated cell-matrix interactions are essential for osteoblast function (Cheng et al., 2000). Increased expression is observed in several invasive malignant cells and in tumour endothelia. αvβ3 integrins may play an important role in the pathogenesis of osteoporosis (Shimaoka and Springer, 2003). The αvβ3 receptors recognize a wide range of extracellular matrix ligands that are similar to those recognized by αIIbβ3.

The structure of β3 integrins

Integrins are αβ heterodimers, consisting of a head domain from which emerge two legs, one from each subunit (Fig. 1), ending in a pair of single-pass transmembrane helices and a short cytoplasmic tail segment. In the absence of a ligand, bonds between the legs and tails hold the head in an "inactive" conformation that has low affinity to ligands (Vinogradova et al., 2002). During "outside-in" signalling, ECM binding to the head triggers conformational changes that are propagated down the "legs" and through the plasma membrane, leading to a separation of the C-terminal fragments, allowing them to bind intracellular proteins (e.g. talin, focal adhesion kinase – FAK) (Giancotti and Ruoslahti, 1999). During "inside-out" signalling, cytosolic proteins bind and sequester one of the cytoplasmic tails, triggering conformational changes in the head that lead to a high-affinity "active" integrin. The heterodimers are formed by the noncovalent association of α and β subunits. In mammals, nineteen α and eight β isoforms have so far been identified, which assemble into 24 different heterodimers (Humphries, 2000). Each α binds only a limited number of β and each αβ has specific ligand-binding properties (Haas and Plow, 1994). In the crystal structure of the extracellular portion of the αA-lacking integrin αvβ3, the "head" comprises a seven-bladed β propeller from the αv subunit that makes intimate contact with a GTPase-like domain of the β subunit (called either an A or I domain). The A domain contains a ligand-binding site called MIDAS (metal ion-dependent adhesion site) in which a metal ion is coordinated by three loops from the A domain, and a glutamic or aspartic acid from the ligand completes an octahedral coordination sphere around the metal. The remaining domains of the two subunits form a pair of legs that come in contact with each other along their lengths, ending at their closely opposed C-termini. The αv tail is composed of three β-sandwich domains: an Ig-like "thigh" domain and two "calf" domains. The β3 tail is built of a PSI (plexins, semaphorins, integrins) domain, four epidermal growth factor (EGF) domains, and a β-tail domain (βTD) (Xiong et al., 2001).

The ligands for β3 integrins

Integrin interactions with ECM proteins are mediated by brief oligopeptide recognition sequences, as proved by experiments with synthetic peptides that can inhibit integrin binding to the matrix (Kraft et al., 1999). The sequences containing RGD and RGD-like motifs are key to β3 integrin interaction. Such sequences in integrin ligands are frequently flexible and often occur in loop regions (Kodandapani et al., 1995). Natural ligands for β3 integrins are presented in Table 1. All these ligands have an RGD or RGD-like motif that is exposed in the central part of their receptor-binding site. The crucial recognition motifs for αIIbβ3 are RGD-like ones: KGD and KQAGDV (Shimaoka and Springer, 2003).

Studies on RGD-motif-containing peptides or peptidomimetic compounds suggest that their integrin-binding specificities depend on the specific conformation, the relative orientation of the side chains

Table 1. Natural ligands for β3 integrins

Integrin type	**Natural ligands**
αIIbβ3	collagen, denatured collagen, decorsin, disintegrins, fibronectin, fibrinogen, plasminogen, prothrombin, thrombospondin, vitronectin, Von Willebrand factor, *Borrelia burgdoferi*
αvβ3	adenovirus penton base protein, bone sialoprotein, cytotactin, denatured collagen, disintegrins, fibronectin, fibrinogen, HIV Tat protein, laminin, matrix metalloproteinase-2, osteoponin, prothrombin, thrombospondin, Von Willebrand factor, vitronectin, *Candida albicans*

of Arg and Asp, and the location of RGD in turn (Li et al., 2003).

Residues flanking the RGD motif also play a critical role in integrin binding specificity. Studies with phage-display peptide libraries indicate that peptides with different sequences flanking the RGD motif show varying degrees of specificity for different integrins.

Sequences surrounding the RGD motif of ligands can tell us about the evolutionary and biological processes that define a given integrin-ligand complex. There are two possibilities: i) the RGD-containing protein can evolve to maximize the overall affinity, or ii) the affinity is fine-tuned to allow for an appropriate biological function (Li et al., 2003).

Disintegrins represent a family of low-molecular-weight, cysteine-rich, RGD-containing peptides that inhibit fibrinogen binding to αIIbβ3 integrin as well as binding of other ligands to RGD-dependent αβ glycoprotein complexes on the surface of other cells. Disintegrins occur naturally in the venom of various vipers, ticks and leeches, and in sperm proteins involved in sperm-egg fusion (Soszka et al., 1991; Niewiarowski et al., 1994; Kang et al., 1998; 1999; Yeh et al., 1998; 2001; Hong et al., 2003).

Propagation of the intracellular signal

The cytoplasmic tails of β3 integrins are short and devoid of enzymatic activity; thus, association with cytoplasmic adapter proteins mediates the transduction of signals. This connection links ECM with cytoskeletal and signalling proteins. Integrins activate many protein tyrosine kinases (FAK, Src-family kinases and Abl), serine/threonine kinase, and integrin-linked kinase ILK (Guan and Shalloway, 1992; Wary et al., 1996). Most integrins can activate the FAK pathway. FAK may be recruited to focal adhesions through a direct and/or talin- and paxilin-mediated interaction with the cytoplasmic tail of integrin β subunits (Chen et al., 1995). When activated, FAK autophosphorylates its Tyr^{397}, which enables SH2 domain binding of Src and Fyn. The Src kinase then phosphorylates other focal adhesion components (e.g. paxilin, tensin, $p130^{CAS}$). In addition, FAK is able to activate PI-3-kinase directly or through the Src kinase (Chen et al., 1996). It was also shown that Src can phosphorylate FAK at Tyr^{925}, creating a binding site for the Grb2/SOS complex, thus linking FAK with mitogen-activated protein kinase (MAPK) cascades (Schlaepfer et al., 1994).

Integrins not only signal on their own, but also cooperate with growth factor receptors (GFRs) in regulating many cellular processes. Integrins appear to associate preferentially with GFRs. It has been shown that αvβ3 forms complexes with the receptors for insulin, platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF) (Schneller et al., 1997; Woodard et al., 1998; Soldi et al., 1999).

The role of β3 integrins in cancer

Integrins are able to mediate adhesive events during various cancer stages such as malignant transformation, tumour growth and progression, invasion and metastasis.

A cell phenotype that results from malignant transformation may contain several alterations in cell adhesion receptors. Altered expression of various integrins during tumour growth and progression has often been described. In some cases, a reduced level of integrin expression has been reported (Miziejewski, 1999). Nevertheless, an overexpression of β3 integrins generally appears to be positively correlated with tumorigenicity. For example, expression of the β3 integrin subunit in melanoma *in situ* has been found to correlate with tumour thickness, the ability to invade and metastasize, and poor prognosis (Marshall and Hart, 1996; Miziejewski, 1999; Trikha et al., 2002). Transition from the radial to the vertical growth phase is a critical step in melanoma progression and survival and is distinguished by the expression of β3 integrin. Moreover, induction of the β3 integrin subunit causes conversion of cancer cells to the vertical growth phase (Marshall and Hart, 1996). Substantial expression of β3 integrins has been observed in various cancer cell lines, e.g. melanoma, glioblastoma, renal carcinoma, ovarian cancer, osteosarcoma, colorectal and breast cancer (Marshalland Hart, 1996; Timar et al., 1998; Miziejewski, 1999). The integrin αIIbβ3 was initially believed to be expressed only in cells of megakaryocytic lineage (e.g. platelets). Later, its presence was detected on tumour cells (Trikha et al., 1996). There is an interesting report describing the Leu33Pro polymorphism of the β3 subunit that modulates the function of αIIbβ3 integrin in human melanoma. According to this report, individuals homozygous for the polymorphism have an increased cancer risk (Bojesen et al., 2003). This shows the important role not only of the level of integrin expression, but also of its molecular characteristics.

The expression of β3 integrins is mostly associated with the ability of tumours to metastasize. Metastasis is a process in which cancer cells detach from the primary tumour, enter the circulation (intravasation), and colonize (extravasation) at distant sites. It is clear that tumour cells can migrate effectively on ECM substrates, and that multiple integrin functioning contributes to this process. Cell adhesion via receptor clustering is required so that cells can put themselves along a migration path (Miziejewski, 1999). This corresponds with findings that β3 integrins can mediate the migration of various cells on several substrates, including vitronectin, fibronectin, fibrinogen, laminin, osteopontin, and collagen (Marshall and Hart, 1996). In fact, studies on murine melanoma, breast cancer, and lymphoma cells showed a positive correlation of substantial αvβ3 expression with the cells' ability to adhere and migrate, thus increasing their metastatic potential. These observations were made in *in vivo* as well as *in vitro* (Matrigel) studies (Timar et al., 1996; Miziejewski, 1999; Li et al., 2001; Kato et al., 2002). Factors that interfere with β3 integrin action abrogated the integrin αvβ3-mediated adhesion and migration of cancer cells (Miziejewski, 1999; Kato et al., 2002).

Although there are far fewer reports of substantial expression of αIIbβ3 (than of αvβ3) integrin on tumour cells, there is an observation that indicates its important role in tumour growth. A study on human melanoma biopsies showed that αIIbβ3 expression increased with tumour thickness, which is indicative of metastatic potential, and this expression increased the ability of melanoma cells to adhere, spread and migrate on fibrinogen. Nevertheless, $\alpha IIb\beta 3^+$ cells had a decreased ability to attach, spread and migrate on vitronectin. Immunocytochemistry showed that expression of αIIbβ3 displaced αvβ3 from focal contact points (Trikha et al., 2002). Studies on non-megakaryocytic lineage B16a cells suggest that αIIbβ3 is constitutively expressed in a high-affinity state, and that this conformation participates in tumour cell adhesion and invasion. High-affinity αIIbβ3 is associated with the Golgi complex and the cell surface. Stimulation of B16a cells induced translocation of the high-affinity integrin from an intracellular pool to the plasma membrane, which resulted in increased tumour cell adhesion to fibronectin (Timar et al., 1998).

Tumour-induced platelet aggregation, which is αIIbβ3-dependent, has been described as a required component (an early step) of metastasis. Tumour cells in vasculature are frequently observed in complexes with platelets. This appears to be essential for successful metastasis. This effect is thought to result from direct binding of platelets to tumour cells. It was reported that β3 receptors, together with ADP, play a crucial role in tumour-induced platelet aggregation and that the disintegrins (see above) may block this stage of metastasis (Oleksowicz et al., 1995; Miziejewski, 1999).

The process of invasion involves both the adhesion and partial proteolytic digestion of the basement membrane layers, followed by cell penetration. β3 integrins are considered to be involved in the regulation of ECM-degrading proteases activity (Marshall and Hart, 1996). Interestingly, αvβ3 integrin colocalizes with the matrix metalloproteinase 2 – MMP2 (gelatinase A, type IV collagenase) on the surface of invasive melanoma cells. This facilitates tumour cell invasion (Miziejewski, 1999). MMP2 is modulated via differential expression of αvβ3 and α5β1 integrins during human melanoma cell invasion (Seftor et al., 1993).

It should also be mentioned that αvβ3 integrin is not essential for metastasis formation. Some cell lines are αvβ3-negative but tumorigenic and able to metastasize (Boukerche et al., 1994; Danen et al., 1995). Further, there are reports that describe quite opposite relations. Human ocular/uveal melanomas preferentially metastasize to the liver by dissemination of the cells via the direct haematogenous pathway. The less invasive uveal melanoma cells express higher levels, while the more invasive cell lines express reduced levels of the αvβ3 integrin (Felding-Habermann et al., 1992; Marshall and Hart, 1996; Seftor, 1998). In primary colorectal cancer, the vascular integrin β3 level in lung metastases was significantly diminished compared with primary tumours or liver metastases (Sato and Miwa, 2002). These observations seem to be inconsistent with most findings. However, progression leading to metastases may require changes in the integrins that would facilitate their ability to leave the primary tumour, and aid in their ability to invade and ultimately form metastases. It is also conceivable that the αvβ3 integrin is reexpressed during various stages of metastatic dissemination and, in particular, during tumour reestablishment (Seftor, 1998).

β3 integrins are also known to play an important role in tumour-induced angiogenesis and have been described as pro-angiogenic factors (Marshall and Hart, 1996; Leu et al., 2002; Nam et al., 2003). Angiogenesis might be defined as the initiation and control of capillary growth. The increased mass of the developing tumour requires continual neovascularization since cell proliferation requires continuous supplies of both oxygen and nutrients. Importantly, a significant role of αvβ3 integrin seems to relate not to its expression by neoplastic cells themselves, but rather to its expression by host endothelial cells. Vascular cell αvβ3 integrin has been implicated in neovascularization and tumour-induced angiogenesis. Importantly, differential expression of αvβ3 integrin was found on newly formed vessels but not on pre-existing vessels (Marshall and Hart, 1996; Miziejewski, 1999). Some reports support the hypothesis that platelets contribute to tumour-induced angiogenesis. In addition, they may explain the clinical observation of an increased platelet turnover in cancer patients (Verheul et al., 2000). It is also known that antagonists of αvβ3 inhibit angiogenic processes (including endothelial cell adhesion and migration) and factors that increase αvβ3 integrin expression and thereby induce angiogenesis (Minamiguchi et al., 2001; Nikos et al., 2002). Nevertheless, the mechanism of inhibition of angiogenesis caused by αvβ3-blocking agents appears to be related with the induction of apoptosis in the activated endothelial cells (Marshall and Hart, 1996). To the best of our knowledge, αIIbβ3 integrin is not engaged in neovascularization.

As the β3 integrins (αIIbβ3 and αvβ3) are so important for tumour progression, they have often been proposed as potential targets for cancer diagnosis and therapy. Several reports show anti-tumour activity of β3 integrin ligands: anti-β3 antibodies or small peptides. The peptides are usually RGD-containing or RGD-mimicking oligonucleotides (Chen et al., 1997; Rahman et al., 2002; Casey et al., 2003; Tucker, 2003).

In summary, β3 integrins appear to have an important, stimulatory role in tumour progression and metastasis. Investigation of the roles of αvβ3 and αIIbβ3 in cancer disease will probably enable the development of some new therapeutic approaches which, it is hoped, will be effective in cancer treatment.

The role of β3 integrins in various diseases

Dysfunction of β3 integrins is implicated in the pathogenesis of some cardiovascular diseases, bleeding disorders (Glanzmann's thrombasthenia), and osteoporosis (Shimaoka and Springer, 2003).

The pathogenesis of thrombotic cardiovascular diseases, such as ischaemic heart disease and stroke, is αIIbβ3 integrin-dependent. Binding of a specific ligand to the platelet integrin αIIbβ3 is a prerequisite stage for platelet thrombus formation. Accordingly, αIIbβ3 integrins have been a prominent target for drug development. There are many types of antagonists available (e.g. cyclic peptides based on the RGD or related amino acid motifs, RGD-based peptidomimetics, and the monoclonal antibody Fab fragment abciximab (Bennett, 2001)).

Glanzmann's thrombasthenia is an exceptional, genetically heterogeneous, autosomal, recessive syndrome associated with a bleeding tendency (Andre et al., 2002). A patient's platelets are characterized by a complete lack of aggregation due to a defect in the αIIbβ3 complex or to a functional abnormality of this integrin (Bellucci and Caen, 2002). The identified defects in genes encoding αIIb or β3 subunits cover the range of known mutations, including gene deletions or rearrangements, nonsense and missense mutations and frameshifts. Abnormalities in the messenger RNA splicing stage are also possible (Basani et al., 2000). As a consequence of these mutations, the biogenesis or conformation of αIIbβ3 integrin is perturbed and the receptor expression on the cell surface is decreased and/or the ability for ligand binding is eliminated (Tozer et al., 1999; Mitchell et al., 2002). Depending on the type and place of the mutation, the impact on severity varies (Ghosh et al., 2002).

The αvβ3 integrin is known to be critical for osteoclast formation and activity (Nemeth et al., 2003). This study was designed to examine the role of αvβ3 expressed by cells native to the bone in the growth and pathogenesis of prostate cancer bone metastases.

The role of β3 integrins in transplant rejection

Recently, evidence has accumulated indicating that β3 integrins play an important role in allograft rejection. Chronic allograft rejection was demonstrated to be associated with a selective increase in αvβ3 expression, which was paralleled by increased synthesis of growth factors as well as ECM proteins. Transfection of COS cells with TGF-β1 caused a selective, almost 4-fold upregulation of that integrin (Jiang et al., unpublished data). This report highlights the role of the integrins in transplant rejection and its association with growth factors; moreover, the selective upregulation of αvβ3 deserves special attention.

Recent data obtained in a clinical transplantation study fully confirm the relevant role of β3 integrins in rejection: marked upregulation of αvβ3 was found in lymphocytic infiltrates and endothelium in biopsies from human heart recipients. This may suggest that αvβ3 plays an important role in the adhesive interactions associated with acute rejection (Yamani et al., 2002b). Further studies demonstrated overexpression of αvβ3 in transplant vasculopathy, a serious post-transplant complication resembling atherosclerosis (Yamani et al., 2002a). Thus, αvβ3 may be the major integrin responsible for cell-cell and cell-ECM interactions leading to acute and chronic transplant rejection with subsequent graft vasculopathy.

β3 integrins and viral infections

Integrins seem to be the "doors" for viruses to enter the cell. The interaction between a virus and integrins plays a key role in the virus multiplication cycle. This interaction brings about membrane permeabilization, fusion, and endocytosis. There are different complex strategies of integrin-dependent virus infectivity. Viruses are able to bind to integrins using pattern recognition sequences that are important for natural ligands (such as RGD tripeptide), or interact with unique regions of integrins without necessarily having a recognition sequence.

β3 integrins are often used as receptors by many different viruses (such as *Adenoviridae, Picornaviridae,*

Table 2. β3 integrins used by viruses for cell entry

Virus	Family	Integrin	References
Adenovirus	*Adenoviridae*	αvβ3	Wickham et al., 1993; Hippenmeyer et al., 2002
Coxackievirus A9	*Picornaviridae*	αvβ3	Roivainen et al., 1994; Triantafilou et al., 1999; Triantafilou et al., 2000
Echovirus 9	*Picornaviridae*	αvβ3	Zimmerman et al., 1997; Nelsen-Salz et al., 1999
Foot and mouth disease virus	*Picornaviridae*	αvβ3	Berinstein et al., 1995; Duque et al., 2003
Hantaviruses	*Buriyaviridae*	αvβ3, αIIbβ3	Gavrilovskaya et al., 1999
Human parechovirus 1	*Picornaviridae*	αvβ3	Triantafilou et al., 2000
Human immunodeficiency virus 1	*Retroviridae*	αvβ3	Lafrenie et al., 2002
Rotaviruses	*Reoviridae*	αvβ3	Guerrero et al., 2000

Buriyaviridae, Papoviridae, Retroviridae, Reoviridae) in their infectious cycle (Table 2). Adenovirus attachment to cells is mediated by a 400 kDa pentavalent subunit (penton base) that contains five RGD sequences and also by a 186 kDa fibre protein. The adenovirus cell entry mechanism depends on the virus type. Most adenoviral infections involve sequential interactions of the virus with host cell receptors, but there are viable adenoviruses 40 and 41 (human subgroup F) that do not carry the RGD motif. This suggests that these viruses can interact with a unique integrin region that does not require the RGD recognition sequence (Triantafilou et al., 2000).

Attachment and entry of the Coxsackie virus A9 (CAV-9) to GMK (green monkey kidney) cells were previously shown to be dependent on the RGD motif in the capsid protein VP1, suggesting integrins as candidate receptors for the virus. This was confirmed in experiments with antibodies specific to the αv and/or β3 integrin subunits that have shown protection of GMK cells from CAV-9 infection (Roivainen et al., 1994). Triantafilou et al., however, have shown that the RGD motif is not an absolute requirement for CAV-9 attachment to the integrin αvβ3 ligand-binding pocket. The CYDMKTTC sequence (187-193 residue) of the integrin was confirmed to be an important binding site for Coxackie virus A9 (Triantafilou et al., 2000).

The echovirus 9 strain Barty (E9/Barty) encodes the RGD motif in the C-terminus of the capsid protein VP1, in contrast to the nonpathogenic prototype strain Hill. Zimmermann et al. proved that the pathogenic character of the Barty strain correlates with a 310-aa segment including the RGD motif. By mutating the RGD to an RGE tripeptide, the infectivity of the resulting echovirus 9 clones for GMK cells was lost. It is also known that the echovirus E9/Barty binds its target cells via contact of the RGD motif with the αvβ3 integrin, whereas the prototype strain Hill uses a different, still unidentified receptor site (Zimmerman et al., 1997; Nelsen-Salz et al., 1999). Echoviruses can also bind α2β1, which is a receptor molecule for laminin and collagen (Santoro and Zutter, 1995).

The foot and mouth disease virus (FMDV) particle contains a loop with a highly conserved RGD sequence in the capsid protein VP1 (G-H loop). The αvβ3 integrin has been identified as a receptor molecule for FMDV by blocking experiments using RGD-containing peptides or antibodies. Deletions and substitutions of the RGD sequence in this virus also resulted in noninfectious phenotypes. In contrast, the G-H loops of the different viruses do not appear to be involved in this phenomenon (Duque and Baxt, 2003). Jackson et al. have recently shown that αvβ6 and α5β1 RGD-dependent integrins may also potentiate receptors for FMDV (Jackson et al., 2000a, b).

It was reported that β3 integrins mediate the extracellular entry of hantaviruses. The RGD motif does not appear in any hantavirus protein and, in addition, neither RGD-containing integrin ligands nor RGD synthetic peptides are able to block hantavirus infection. The result suggests that hantaviruses associate with integrins through unique regions, or require more complex cell receptor interactions for their entry (Gavrilovskaya et al., 1999).

Human parechovirus 1 (HPEV-1) has the RGD motif in its VP1 capsid protein (Hyypia et al., 1992). By using peptide libraries it has been shown that HPEV-1 uses αv (αvβ3 and αvβ1) receptors in its infectious cycle (Pulli et al., 1997).

Attachment of human immunodeficiency virus type 1 (HIV-1) to macrophages is a critical early event in the establishment of infection. The involvement of integrin αvβ3 in HIV-1 infection of peripheral blood monocyte-derived macrophages has been demonstrated. It was shown that an increasing level of αvβ3 expression was accompanied by increased HIV-1 replication in monocytes. The purified HIV-gp120 protein was able to interact with the αvβ3 integrin receptor and, what is more, that antibody substantially inhibited HIV infection of monocytes.

Rotaviruses have very specific cell tropism of renal or intestinal epithelium origin only. Very often, rotavirus strains attach to sialic acid on cell surfaces, but this process is not essential for virus infectivity. It is known that integrins α2β1, α4β1 and β2 have been implicated in rotavirus cell entry (Coulson et al., 1997). αvβ3 integrins play an important role in a post-binding stage of the rotavirus infectious cycle. These interactions are RGD-independent, because rotaviruses do not express RGD motifs on their surface proteins and rotavirus entry could not be inhibited by RGD peptides (Guerrero et al., 2000).

There is also the hypothesis that bacteriophages (bacterial viruses) use a cellular receptor (β3 integrins) for their attachment to eukaryotic cells. Some phages (e.g., T4) present a KGD (Lys-Gly-Asp) sequence in their external proteins (there are 55 copies of the KGD motif in the head corner protein of the T4 phage) (Gorski et al., 2003a).

Possible relationships between KGD-positive phages and the protein ligand for CD40 molecule (CD40L) seem to be very interesting. CD40L is a surface membrane protein structurally similar to TNFα. It appears in activated T cells as a ligand for CD40. CD40/CD40L plays an important role in both normal immunological response and pathological immune stages (allograft rejection, autoimmune disorders, arteriosclerosis and cancer) (Buchner et al., 2003).

Recently it has been shown that a strong immunosuppressive effect may be obtained with appropriate antibodies, among others by elimination of activated T lymphocytes (Waldmann, 2003). CD40L is also expressed in platelets, but its activity appears after specific activation. CD40L is a platelet agonist phosphorylating its main integrin, αIIbβ3, and consequently, inducing platelet aggregation and thrombogenesis (Andre et al., 2002). An increase in the circulating CD40L level has been shown in patients with Crohn's disease and with infiltrating colon inflammation (Danese et al., 2003). This stage also accompanies the process of restenosis in arteriosclerosis patients that have undergone surgical resorting of arterial patency of coronary arteries (Cipollone et al., 2003) and indicates an increased risk of vascular incidents in women (Varo et al., 2003).

It has also been shown that CD40L may function as a proinflammatory and proangiogenic factor (Reindes et al., 2003). These extremely important interactions of CD40L seem to be connected with the presence of the KGD amino acid motif in its molecule. This tripeptide sequence binds to a platelet's integrin αIIbβ3 (Prasad et al., 2003). It has been shown that αIIbβ3 antagonists inhibit CD40L production (Prasad et al., 2003). As was already mentioned, CD40L plays an extremely important role in arteriosclerosis and its cardiovascular complications, inflammations, allograft rejection and in cancer disease. In this light, the discovery that the T4 phage head protein contains a KGD sequence (probably responsible for its immunomodulatory effects) gives new and exciting possibilities for clinical applications of the reciprocal interactions and/or competition, as we already suggested in our hypothesis about new insights into the role of bacteriophages in nature and in the defence of higher organisms (Gorski et al., 2003a). Our preliminary results showing an immunosuppressive effect of KGD-positive T4 phages seem to confirm this hypothesis (Gorski et al., 2003b).

Murine models for studies on integrin functions *in vivo*

Studies utilizing integrin knockout mice and cells derived from these mice have provided considerable and sometimes surprising insights into the unique functions of individual members of the integrin family. Thus far, mice expressing null mutations of seven of the eight β subunits and 13 of the 18 known α subunits have been generated. With only a few exceptions, the phenotypes of each of the knockout lines are quite distinct (Sheppard, 2000).

Experiments with β3-integrin-deficient mice have suggested that β3 integrins play critical roles in diverse biological processes including embryo implantation, angiogenesis, and wound healing. Mice lacking the β3 subunit are a good model for the human bleeding disorder – Glanzmann's thrombasthenia. β3-null mice have virtually all the cardinal characteristics of the human disease, including gastrointestinal and cutaneous haemorrhage, prolonged bleeding times, abnormal platelet aggregation and clot retraction. β3-null mice also have an abnormality in osteoclast function that leads to a gradual accumulation of osteoids and osteosclerosis (Hodivala-Dilke et al., 1999; McHugh et al., 2000).

Ablation of the gene for the αv integrin subunit in mice, though this leads to death, allows considerable embryonic development and organogenesis, including extensive vasculogenesis and angiogenesis. These mice are able to survive until late in embryonic development and occasionally even to birth. The animals have cleft palates and die from massive CNS (central nervous system) or gastrointestinal haemorrhage, which appears to result from a defect in the development of blood vessels in these organs (Bader et al., 1998).

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