Cellular Receptors for Foot and Mouth Disease Virus

J. Ruiz-Sáenz^{a, b} Y. Goez^c W. Tabares^a A. López-Herrera^d

^aGrupo Inmunovirología-Biogénesis, Sede de Investigación Universitaria, Universidad de Antioquia,

^bGrupo de Microbiología y Epidemiología, Universidad Nacional de Colombia, ^cInstituto Colombiano de Medicina Tropical/Universidad CES, and ^dGrupo BIOGEM, Universidad Nacional de Colombia, Medellín, Colombia

Key Words

Foot and mouth disease virus • Virus receptors • Integrins • Heparan sulfate (source: MESH)

Abstract

Foot-and-mouth disease virus (FMDV), the prototype member of the *Aphthovirus* genus, is a single-stranded, positive-sense RNA genome virus, which affects many domestic livestock cloven-hoofed animals, causing substantial loss of milk in dairy cattle, reduction in the growth rate of meat animals, among others. It has been shown that the virus can enter to the cells using different pathways; the main one binding integrins via the clathrin-mediated endocytosis pathway, trafficking throughout the acidified endocytic vesicles, where its capsid rapidly dissociates, resulting in the release of the RNA genome, and the second one using heparan sulfate in which FMDV enters to the cells using the caveola-mediated endocytosis pathway and that caveolae can associate and traffic with endosomes. Different integrins had been involved as FMDV receptors ($\alpha v\beta 1$, $\alpha v\beta 3$, $\alpha 5\beta 1$, $\alpha v\beta 6$, $\alpha v\beta 8$); this review will try to resume the basic information about FMDV receptors from the last years to the present and will resume the most important in vitro and in vivo studies to elucidate the role of this receptor on the infection.

Copyright © 2009 S. Karger AG, Basel

Introduction

Foot and mouth disease virus (FMDV) is the prototype member of the *Aphthovirus* genus of the *Picornaviridae* family. There are seven serotypes: A, O, C, Asia-1, and South African Territories 1 (SAT1), SAT2, and SAT3, but a large number of subtypes have evolved within each serotype [1–3]. FMDV is a small, non-enveloped, icosahedral virus with a single-stranded, positive-sense RNA genome of approximately 8,400 nucleotides [1, 4]. The viral capsid is made up from 60 copies each of four viral-encoded proteins, VP1 to VP4; VP1, VP2 and VP3 form the outer capsid shell, and VP4 makes up the interior surface [1]. An unusual structural feature of the outer capsid surface is a long, conformationally flexible loop of VP1. This loop (the G-H loop) forms a major antigenic site on the virion and it includes in its apex an Arg-Gly-Asp (RGD) motif [5, 6], a sequence that has been implicated in receptor binding on the basis of synthetic peptide inhibition studies [7].

The virus affects many domestic livestock such as pigs, sheeps, goats, and cattle, all of them cloven-hoofed animals and *Camelidae* (i.e. members of the order *Artiodactyla*). Those animals are susceptible to infection with FMDV, but the degree by which different species and even breeds of animals develop clinical disease varies [8, 9].

The primary route of infection is through the upper respiratory tract, with a strong tropism for epithelial cells. Following a natural infection in ruminants, initial viral replication is thought to take place in epithelial cells of the oro-pharynx. During the development of disease, virus is widely disseminated throughout the body with secondary replication sites in many epithelial tissues [8, 10].

Foot and mouth disease (FMD) is one of the most economically important veterinary diseases due to its highly infectious nature, and its ability to cause persistent and long-term effects on the condition and productivity of many animal species; countries which have the disease have many trade restrictions placed upon them [3]. The infection of livestock invariably results in: substantial loss of milk yield in dairy cattle, reduction in the growth rate of meat animals, temporary loss of draught power with working buffalo and cattle, reductions in fertility, and death in younger animals. Additionally, the incidence of FMD results in the need to cull unprotective and chronically infected animals, gross disruption of farming practices including loss of income, loss of valuable breeding stock and disruption of livestock improvement programs [11, 12].

FMDV enters the cell by receptor-mediated endocytosis, in a process that begins with the initial attachment of the virus to cell-surface receptors. Once the virus is inside the endosome, the low pH of this compartment triggers uncoating of the viral genome, which is then translocated across the endosomal membrane into the cytosol [13, 14]. This review focuses on the molecules that have been cited as possible receptors for FMDV and attempts to summarize the research in this field.

Integrins

Integrins are proteins of crucial biological importance that are used by cells to bind and respond to the extracellular matrix. They belong to a large family of integral membrane receptors that function as cell adhesion molecules. Functional integrins consist of two noncovalently bound transmembrane glycoprotein subunits designated alpha (α) and beta (β) [16]. Each subunit is composed of a large extracellular domain of >940 (α) and >640 (β) residues (120–180 and 90–110 kDa, respectively), a single transmembrane domain and a small, C-terminal cytoplasmic domain [16]. The cytoplasmic tails are short (10–50 residues) and they do not contain any catalytic activity or consensus protein-binding motifs, except for the

internalization NPXY motif in β subunits. The exception is the $\beta 4$ subunit with two fibronectin type III repeats in its uniquely long cytoplasmic domain (1,000 amino acids). The α and β subunits are all homologous to each other [17, 18], and both subunits bind the specific ligand. To date, 19 α and 8 β subunits have been identified, meaning that in theory, the different subunits could associate to give more than 100 distinct integrin heterodimers. However, from these subunits approximately 24 integrins have been found in nature, because many α subunits can associate with only a single β subunit. For example, the $\beta 4$ subunit can only form a heterodimer with the $\alpha 6$ subunit, while the $\beta 1$ subunit can form heterodimers with ten different α subunits. The $\alpha/\beta 1$ heterodimers do not have the same ligand specificities; it is believed that the α chain is, at least partly, involved in the ligand specificity. The diversity of the integrins is enhanced by alternative splicing of certain integrin messenger RNAs [19, 20].

The functions of individual integrins have been elucidated using cell adhesion assays, monoclonal antibodies, and affinity chromatography. Integrins contribute to a variety of processes including: (1) adhesion between cells, and between cells and the extracellular matrix; (2) induction of signal transduction pathways that modulate various processes, including cell proliferation, morphology, migration and apoptosis; (3) interaction with many extracellular ligands participating in a large range of physiological processes, including embryogenesis, morphogenesis, wound repair, inflammation, tumor cell migration and leukocyte trafficking. Their ligands include extracellular matrix proteins such as fibronectin, collagen, laminin, other cell surface receptors that are members of the immunoglobulin superfamily (e.g. intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1) and soluble proteins like the fibrinogen, inactivated complement component C3 (iC3b) [21–24]. In addition, integrins take part in physiological processes such as platelet aggregation, a classical example of a process dependent on integrin affinity modulation, because specificity and affinity of given integrin receptors on a given cell are not always constant [19, 25].

Integrins can bind to an array of ligands; the binding of ligands to integrins is universally bivalent cation dependent, thus, it is important to consider the location, specificity and functional role of cation-binding sites. Integrin-ligand binding is usually stimulated by Mg^{2+} and Mn^{2+} , and inhibited by Ca^{2+} [26]. To adhere to ligands, both subunits, α and β , of the integrin are needed as well as divalent cations that bind to the α chain. However, the

substrate specificity of the integrins depends upon the cell type on which it is expressed [27].

Integrins differ from other cell-surface receptors in that they bind their ligands with low affinity (10^6 – 10^9 liters/mol) and that they are usually present at 10- to 100-fold higher concentrations on the cell surface than other surface receptors [20]. The integrins, however, can only bind their ligands when they exceed a certain minimal number in given places, known as focal contacts or hemidesmosomes. When the integrins are distributed diffusely over the cell surface, the adhesion cannot occur; however, when certain stimuli are present, the integrins cluster to form focal contacts. Their combined weak affinities give rise to a spot on the cell surface with enough adhesive capacity to bind to the extracellular matrix [24]. This very advantageous situation allows cells to bind simultaneously but weakly to large numbers of matrix molecules and still have the opportunity to explore their environment without losing attachment by building or disrupting focal contacts. If the receptors were to bind strongly to their ligands, cells would probably be irreversibly bound to the matrix, depriving it from motility. Such a problem does not arise when attachment depends on multiple weak adhesions [24]. The integrins, in cell adhesion, deliver 'outside-in' signals that control cell proliferation, gene induction, survival, differentiation, and cell motility. To achieve these diverse biological outcomes, integrins are recruited in complexes that contain numerous cytoskeletal proteins and signaling molecules. The functions of integrins themselves are regulated by 'inside-out' signaling [28].

The α subunits have very similar structures; they contain seven homologous repeats of 30–40 amino acids in their extracellular domain, spaced by 20–30 amino acid stretches. The most extracellular three or four repeats contain sequences with cation-binding properties thought to be involved in cation-dependent integrin-ligand binding. All α subunits share the 5 amino acid motif GFFKR, located directly below the transmembrane region. The precise function of this motif is not known, but calreticulin, a calcium storage protein, has been isolated on a column loaded with this fragment. Moreover, an association between calreticulin and the integrins α -3 and α -5 has been demonstrated. Calreticulin may play a role in the assembly of integrin dimers [29].

The α subunits are subdivided into two groups based on certain structural differences. The first group is formed by α -1, α -2, α -L, α -M and α -X. The members of the first group all contain an inserted domain (I-domain). This domain of about 180 amino acids is situated between

the second and the third repeats. The function of this domain is not known, but it has been speculated that it is involved in ligand binding, because the domain resembles those domains found in von Willebrand factor that are important for the binding to collagen. The second group is formed by α -3, α -5, α -6, α -7, α -8, α -IIb, α -V and α -IEL. Members of this group all undergo post translational cleavage of their precursor into a heavy and a light chain. The light chain is composed of the cytoplasmic domain, the transmembrane region and a part of the extracellular domain (about 25 kD), while the heavy chain contains the rest of the extracellular domain (about 120 kD) [29].

The β subunit has an N-terminal region that contains a 90-residue repeat termed a PSI domain (for plexins, semaphorins and integrins). The tertiary structure of this module has not yet been solved, but it has been predicted to behave like an α -helix [29]. The PSI domain co-operates with a more C-terminal cysteine-rich region in restraining the integrin in an inactive state via a long-range disulfide bond. The β subunits also contain an I-domain with a metal ion-dependent adhesion site (MIDAS) and similar secondary structure as the I-domain of the α subunits. When associated with an α subunit lacking an I-domain, this I-domain-like structure takes direct part in ligand binding, where it otherwise would have a more indirect regulatory effect. This domain is also involved in the actual association of the β subunit with the α subunit, and it is mutually dependent on the β propeller for correct folding. As with the α subunit, there is a stalk region in the C-terminal part of the extracellular domain, but unlike α subunits, this region contains four EGF-like cysteine-rich domains important in signal transduction and activation of the integrin. Six of the eight β subunits show a high degree of similarity in three regions of their cytoplasmic tails, called cyto-1, cyto-2 and cyto-3, important for focal contact localization, and a threonine-serine stretch involved in cell adhesion [18, 19, 29].

Integrins as FMDV Receptors

Several integrins have been identified as FMDV receptors, but most of these studies were done with human integrins. An overview of the principal integrins and other cellular receptors recognized as FMDV receptors and a summary of this research are presented in the next part of this review.

Integrin $\alpha\beta 3$ as a Cellular Receptor for FMDV

Originally identified as a receptor for vitronectin, integrin $\alpha\beta 3$ is now known to recognize the RGD tripeptide in up to nine different proteins ligands. Binding to at this sequence, these ligands are differentially regulated by divalent cations, essential for integrin-ligand interactions [19, 30, 31]. It cations regulate the affinity and specificity of ligand binding most likely by influencing the shape of the binding pocket. The binding of ligands to $\alpha\beta 3$ is usually supported by Mg^{2+} and can be enhanced by Mn^{2+} . In addition, Ca^{2+} supports the binding of many ligands such as vitronectin and fibronectin, but inhibits binding of others such as fibrinogen [32]. Previous studies of FMDV binding to its integrin receptor have been complicated by the abundance of its heparan sulfate coreceptor on the surface of susceptible cells. However, using a purified preparation of human integrin $\alpha\beta 3$ in an enzyme-linked immunosorbent assay, it was demonstrated that viruses representative of all FMDV serotypes bind to $\alpha\beta 3$ in an RGD-dependent interaction [33]. In 2002, Xiong et al. [34] showed that $\alpha\beta 3$ interacts with a divalent-cation-binding site defined by the presence or absence of an A-domain in the α subunit; and the study of RGD- $\alpha\beta 3$ complex structure showed that the peptide makes contact with both subunits, the arginine fitting into a cleft formed by $\alpha\beta$ -propeller loops and the aspartate coordinating the cation in the $\beta 3$ A-domain.

Using baby hamster kidney cells, the integrin $\alpha\beta 3$ has been shown to act as a receptor for the internalization of FMDV serotype A12 [35]. Also, mutational studies with an infectious cDNA clone of the same virus have shown that interaction with integrin is mediated by the G-H loop-containing RGD. This motif appears to be essential for entry, since mutations in this sequence, including the conservative change to RGE, result in noninfectious virus particles that fail to enter cells [36]. In 1997, Leippert et al. [37] obtained similar results using a cloned infectious cDNA of the FMDV subtype O1K. Although most mutations introduced into the RGD motif resulted in virus particles that were deficient only in their ability to enter cells, in contrast to subtype A12, changing the RGD to RGE produced viable virus particles. Nevertheless, the G-H loop-containing RGD is highly conserved among FMDV serotypes, indicating that viruses will enter cells by means of an RGD-binding integrin.

Early experiments performed in COS cell cultures, which are not susceptible to FMDV infection, became susceptible after cotransfection with α v and $\beta 3$ subunit cDNAs from bovine or human sources using engineered bovine-human chimeric $\beta 3$ subunits. Cells cotransfected

with the bovine cDNAs of $\alpha\beta 3$ subunit and then infected with FMDV synthesize greater amounts of viral proteins than did infected cultures cotransfected with the human integrin subunits. Cells cotransfected with a bovine α v subunit and a human $\beta 3$ subunit synthesized viral proteins at levels equivalent to those in cells expressing both human subunits. However, cells cotransfected with the human α v and the bovine $\beta 3$ subunits synthesized amounts of viral proteins equivalent to those in cells expressing both bovine subunits, indicating that the bovine $\beta 3$ subunit is responsible for the increased effectiveness of this receptor. The increase in receptor efficiency is due to sequences encoding the C-terminal one third of the subunit ectodomain, which contains a highly structured cysteine-rich repeat region; in that way, the amino acid sequence differences within this region may be responsible for structural differences between the human and bovine $\beta 3$ subunit, leading to more efficient utilization of the bovine receptor by the virus [38].

Studies by Neff et al. [38] indicate that virulent FMDV utilizes the $\alpha\beta 3$ integrin as a primary receptor to initiate infection of cultured cells and that adaptation of type O1 virus to cell culture results in the ability of the virus to utilize heparan sulfate as a receptor and a concomitant loss of virulence. When normal Chinese hamster ovary cells (CHO), which express heparan sulfate but not $\alpha\beta 3$, and two heparan sulfate deficient CHO cell lines were transfected with cDNAs encoding human $\alpha\beta 3$, a panel of cells was produced that expressed one or both receptors. In these cells, the replication of FMDV type A12 was dependent on the expression of $\alpha\beta 3$, whereas type O1BFS replicated to high titer in CHO cells but could not replicate in heparan sulfate deficient cells, regardless of the expression of $\alpha\beta 3$. Experiments using two genetically engineered variants of FMDV type O1Campos, vCRM4 and vCRM8 indicated that vCRM4 had reduced virulence in cattle and could bind to heparin-Sepharose columns, while vCRM8 did not. VCRM4 replicated in K562 and CHO (which express heparan sulfate⁺ $\alpha\beta 3$ ⁻) cells, whereas vCRM8 replicated only in K562 and CHO cells transfected with $\alpha\beta 3$ cDNAs. A similar result was obtained using a vCRM4 virus with an engineered RGD to KGE mutation [14, 39].

A high degree (96%) of amino acid sequence similarity between the α_v and $\beta 3$ subunits of the bovine integrin and its corresponding human subunits exists in the ectodomains as well as identical transmembrane and cytoplasmic domains. Within the putative ligand-binding domains, the α v subunits from bovine and human have a 98.8% homology in their amino acid sequence, while

there is only a 93% homology between the $\beta 3$ subunits of these two species [38].

A comparison of the amino acid sequences of the bovine and human $\beta 3$ subunit between position 488 and the C terminus reveals virtually identical transmembrane and cytoplasmic domains, with only seven amino acid changes in the ectodomain. This region of the subunit is rich in cysteines, thereby contributing to the overall structure of the integrin through disulfide bonds [40].

Although FMDV can utilize human and simian $\alpha \nu \beta 3$ as receptor in cell culture [14, 39], it is quite clear that the virus replicates efficiently in cells expressing the bovine integrin, specifically its cysteine rich repeat region of the $\beta 3$ subunit of that integrin, which has a high degree of structural conservation among all β subunits [41]. Thus, FMDV has probably evolved into a disease of cloven-hoofed livestock because the structure of the $\alpha \nu \beta 3$ receptors of these animals results in a more advantageous fit with the viral surface, which would in turn lead to much greater viral replication and disease within these species. However, since this integrin probably performs similar functions in a wide variety of species, the structural differences cannot be radically different, as evidenced by the high degree of sequence similarity between the bovine and human integrins [38].

Studies by Jackson et al. [42] using a solid-phase receptor binding assay, characterized the binding by FMDV to purified $\alpha \nu \beta 3$ in the absence of heparan sulfate and other cell surface components. FMDV (O1BFS) efficiently used authentic ligand binding by cellular $\alpha \nu \beta 3$ in terms of its high affinity, dependence on divalent cations, and activation by ions as manganese. Virus binding to $\alpha \nu \beta 3$ was sensitive to competition by short RGD containing peptides (50% inhibition at $<10^{-8}$ M peptide), and the inhibition was highly sequence specific, with the equivalent RGE peptide being at least 10^4 -fold less effective as a competitor. Viruses of the other six serotypes of FMDV bound to $\alpha \nu \beta 3$ in a similar RGD-specific manner, although significant differences in sensitivity to RGD peptides suggest that the affinity of the different FMDV serotypes for $\alpha \nu \beta 3$ is influenced, in part, by the variable amino acid residues in the VP1 G-H loop on either side of the RGD.

In 2001, Neff et al. [43] found that truncations or extensions of the cytoplasmic domains of the $\alpha \nu$ and $\beta 3$ subunits, including deletions removing almost all of the cytoplasmic domains, had little or no effect on the ability of the integrin to function as a receptor for FMDV. The lysosomotropic agent monensin inhibited viral replication in cells transfected with either intact or cytoplasmic

domain-truncated $\alpha \nu \beta 3$. Also, they found that viral replication in transfected cells was inhibited by an antibody blocking the $\alpha \nu \beta 3$ function, but not by function-blocking antibodies to other RGD-directed integrins $\alpha \nu \beta 5$, $\alpha 5 \beta 1$, and $\alpha \nu \beta 6$, suggesting that these integrins are not involved in the infectious process. They demonstrated that alterations to the cytoplasmic domains of either subunit, which lead to the inability of the integrin receptor to function normally, do not abolish the ability of the integrin to bind and internalize this viral ligand.

Studies by Monaghan et al. [44] in 2005 used immunofluorescence confocal microscopy and real-time RT-PCR to determine $\alpha \nu \beta 3$ and $\alpha \nu \beta 6$ expressions within the epithelial cells that are normally targeted by FMDV in cattle. Results showed that $\alpha \nu \beta 6$ but not $\alpha \nu \beta 3$ was expressed on the surfaces of the epithelial cells at the sites where FMDV is known to replicate at a high level during a natural infection. Expression of $\alpha \nu \beta 6$ on the cells targeted by FMDV within the interdigital skin was confirmed by showing the presence of this integrin on the surface of infected cells. Together, these data suggested that, in epithelia normally targeted by FMDV in cattle, $\alpha \nu \beta 3$ does not serve as the major receptor that determines virus tropism.

Integrin $\alpha 5 \beta 1$ and Its Ability as FMDV Receptor

Some integrins, like $\alpha 5 \beta 1$, bind their ligands through recognition of an RGD motif. These integrins are expressed on epithelial and lymphoid cells, where they may serve as cellular receptors for FMDV. Integrin $\alpha 5 \beta 1$ is known to bind this motif; however, despite having an RGD, FMDV appears unable to use all of these integrins to initiate infection and evidence for $\alpha 5 \beta 1$ as receptor has been consistently negative [45–47].

In a study using a solid-phase receptor-binding assay to characterize the binding of FMDV to purified preparations of the human integrin $\alpha 5 \beta 1$ in the absence of heparan sulfate and other RGD-binding integrins, the binding of FMDV resembled authentic ligand binding to $\alpha 5 \beta 1$ in its dependence on divalent cations and specific inhibition by RGD peptides. Most importantly, binding was found to be critically dependent on the conformation of the integrin, as virus bound only after induction of the high-affinity ligand-binding state. In addition, the identity of the amino acid residue immediately following the G-H loop RGD motif has been shown influencing differentially the ability of FMDV to bind integrins $\alpha 5 \beta 1$ and $\alpha \nu \beta 3$. Jackson et al. [45] concluded that viruses that have a leucine immediately following the RGD motif can serve as ligands for both $\alpha 5 \beta 1$ and $\alpha \nu \beta 3$, whereas viruses that

have an arginine at this position become better ligands for $\alpha\beta 3$, but at the expense of becoming poor ligands for $\alpha 5\beta 1$.

Epithelial Integrin $\alpha\beta 6$ Is a Receptor for FMDV

Expression of $\alpha\beta 6$ is restricted to epithelial cells and it has been observed at a variety of sites, including the epithelia of the uterus, bladder, respiratory tract, and salivary glands. Expression levels are moderate or low in normal healthy adult epithelia but are rapidly upregulated at sites of tissue injury and inflammation. In addition, inactivation of the $\beta 6$ subunit in mice has identified a role for $\alpha\beta 6$ in downregulation of inflammation in skin and respiratory tract. Integrin $\alpha\beta 6$ also plays an important role in keratinocyte migration and it is expressed at the leading edges of healing cutaneous wounds. Also, integrin $\alpha\beta 6$ has been shown to confer upon cells a growth advantage, an effect dependent on an 11-amino-acid COOH-terminal extension that is unique to the $\beta 6$ subunit [17].

Jackson et al. [48] studied integrin $\alpha\beta 6$ as a candidate for use as a receptor by FMDV as it is expressed on epithelial cells, which correlates with the tissue tropism of the virus. They showed that cells SW480 from human colon carcinoma which are normally nonpermissive for FMDV become susceptible to infection after transfection with the integrin $\beta 6$ subunit and expression of $\alpha\beta 6$ at the cell surface. Integrin $\alpha\beta 6$ is the major site for virus attachment on the $\beta 6$ -transfected cells, and binding to $\alpha\beta 6$ serves to increase the rate of virus entry into these cells. In this study, the virus binding was inhibited by >90% using a monoclonal antibody (10D5), which specifically recognizes $\alpha\beta 6$ and also inhibits binding of its natural ligands. Also, it was shown that the infection of the $\beta 6$ -transfected cells by FMDV is inhibited by >99% by the same antibody. In addition, it was shown that virus binding and infection of the $\beta 6$ -transfected cells is mediated through an RGD-dependent interaction.

The integrin $\alpha\beta 6$ was shown to function as a receptor for virus attachment; however, it was not known if the $\alpha\beta 6$ receptor itself participated in the events that follow virus binding to the host cell. In 2001, Miller et al. [17] investigated the effects of several deletion mutations in the $\beta 6$ cytoplasmic domain on infection. The authors showed that although loss of the $\beta 6$ cytoplasmic domain has little effect on virus binding, this domain is essential for infection, indicating a critical role in post-attachment events.

Prior to these studies, $\alpha\beta 3$ was the only member of the integrin family that had been shown to act as a recep-

tor for FMDV, but that integrin has limited expression on epithelial cells and cells of lymphoid origin, the cells in which FMDV is likely to reside during the initial phase of infection. By contrast, $\alpha\beta 6$ is expressed exclusively on epithelial cells, including sites where initial virus replication is believed to occur, making this integrin a more likely candidate as the receptor used by FMDV during the initial phase of infection in an animal [17, 35, 48].

In 2005, Berryman et al. [14] and O'Donnell et al. [49] simultaneously reported that the infection of $\alpha\beta 6$ -expressing cells with FMDV takes place in association with the integrin in vesicular structures in a clathrin-mediated endocytosis pathway, followed by acidification within endosomes that facilitates viral replication, causing the breakdown of the viral capsid structure and release of the genome by an as-yet-unidentified mechanism. Moreover, not caveolae or other endocytic pathways that depend on lipid rafts were found [49]. Similar observations were obtained using a CHO cell line transfected with integrin $\alpha\beta 6$, providing evidence for a functional link between the presence of FMDV within endosomes and infection as well as showing that virus binding induces endocytosis of $\alpha\beta 6$ and that the integrin serves to deliver the virus into the early and recycling endosomes [14].

Experiments on cattle and in vitro were performed by Rieder et al. [50] in 2005 to study the pathogenesis of FMD after infection with an A24cru type virus, which contained an SGD receptor recognition site. It was not clear why A24Cru cattle infection selected an SGD variant that utilizes only the $\alpha\beta 6$ integrin in vitro, while the original RGD virus utilizes multiple ($\alpha\beta 1$, $\alpha\beta 3$, $\alpha\beta 6$) integrin receptors. They speculated that it is possible that the route of inoculation and the availability of appropriate integrin receptor molecules on the cells at the portal of entry may be determining this selection. Therefore, it has been reported that $\alpha\beta 6$ integrin is expressed in epithelial cells, the main cell type infected by FMDV in susceptible host [51].

The aforementioned observations strongly suggest that RGD-dependent integrins probably direct FMDV to target tissues during a natural infection in susceptible hosts, the fact that the virus can utilize multiple α integrins in vitro elicits the question of how these different receptors function in determining viral pathogenesis. To answer this question, Duque and Baxt [47] studied molecularly cloned bovine $\beta 1$, $\beta 5$, and $\beta 6$ integrin subunits. Using these subunits, along with previously cloned bovine $\alpha\beta 3$ subunits, in a transient expression assay system, they compared the efficiency of infection mediated

by $\alpha\text{v}\beta 1$, $\alpha\text{v}\beta 3$, $\alpha\text{v}\beta 5$, and $\alpha\text{v}\beta 6$ among three strains of FMDV serotype A and two strains of serotype O. While all the viruses could infect cells expressing these integrins, they exhibited different efficiency of utilization of the integrin. All type A viruses used $\alpha\text{v}\beta 3$ and $\alpha\text{v}\beta 6$ with relatively high efficiency, while only one virus used $\alpha\text{v}\beta 1$ with moderate efficiency. In contrast, both type O viruses used $\alpha\text{v}\beta 6$ and $\alpha\text{v}\beta 1$ with higher efficiency than $\alpha\text{v}\beta 3$. Only low levels of viral replication were detected in $\alpha\text{v}\beta 5$ -expressing cells infected with either serotype. Experiments in which the ligand-binding domains among the β subunits were exchanged indicated that this region of the integrin subunit contributes to the differences in utilization of the integrins among strains. While infection with different FMDV strains may result in different degrees of disease severity, in most cases there are no apparent differences in clinical symptoms within a species; it is possible that these may be related to differences in either expression patterns or degrees of expression of the known integrin receptors for FMDV among different species.

Although it has been demonstrated that FMDV binds to isolated purified $\alpha 5\beta 1$, $\alpha\text{v}\beta 3$ and $\alpha\text{v}\beta 6$ integrins, the role of soluble viral receptors in FMDV infection has not been evaluated. To evaluate this role, COS-1 cells were transfected with plasmids encoding the soluble αv subunit and either the soluble $\beta 3$ or $\beta 6$ subunit and labeled with [35S]methionine cysteine. For examination of the biological activity of integrins, the authors generated stable cell lines producing the soluble integrins in HEK293A cells. In the presence of divalent cations, soluble $\alpha\text{v}\beta 6$ bound to representatives of type A or O viruses, and significantly inhibited viral replication, as determined by plaque reduction assays. In contrast, soluble $\alpha\text{v}\beta 3$ was unable to bind to immobilized virus of either serotype; however, virus bound to the immobilized integrin, suggesting that FMDV binding to $\alpha\text{v}\beta 3$ is a low-affinity interaction. In addition, soluble $\alpha\text{v}\beta 3$ did not neutralize virus infectivity. Incubation of soluble $\alpha\text{v}\beta 6$ with labeled type A12 or O1 resulted in a significant inhibition of virus adsorption to BHK cells, while soluble $\alpha\text{v}\beta 3$ caused a low, but consistent, inhibition of virus adsorption, showing that soluble receptors can interfere with FMDV infection, at least in vitro, and providing basis to analyze the use of soluble receptors and anti-integrin compounds as a FMD control strategy [52].

Monaghan et al. [15] found that $\alpha\text{v}\beta 6$ expression had a good correlation with the abundance of $\beta 6$ mRNA at the sites of lesions in cattle. The tongue and interdental skin are sites where FMDV replicates to a high level and

lesions normally form [8]. At these sites, $\alpha\text{v}\beta 6$ protein was readily detected in the tissue and $\beta 6$ mRNA was more abundantly expressed than that of $\beta 3$. Within these epithelia, the highest levels of $\alpha\text{v}\beta 6$ were present on the surfaces of epithelial cells of the stratum spinosum, the cells targeted by FMDV in cattle. Similarly, these cells are targeted in the tongue of FMDV-infected pigs [8, 15, 44].

The integrin $\alpha\text{v}\beta 6$ was also detected on surfaces of epithelial cells within the coronary band and ventral soft palate. However, at these sites, $\alpha\text{v}\beta 6$ showed greater variation between animals and was detected in 3 of 4 and 2 of 6 animals in the coronary band and ventral soft palate, respectively. The variable expression of $\alpha\text{v}\beta 6$ at these sites could result from differential integrin expression between cattle or, alternatively, from nonuniform expression of $\alpha\text{v}\beta 6$ within the epithelium of individual animals. It could result from upregulation of $\alpha\text{v}\beta 6$ at sites of inflammation. The variable expression of $\alpha\text{v}\beta 6$ protein within the coronary band and ventral soft palate is, however, consistent with the frequency of lesions at these sites. Lesions are often but not always present on the coronary band and rarely seen on the ventral soft palate. Although it is likely that FMDV infection (and hence lesion formation) requires a number of cellular factors, it is interesting to speculate that lesion formation at these sites is dependent on expression of $\alpha\text{v}\beta 6$ [44].

Recent studies developed by Brown et al. [53] reported the cloning and sequencing of ovine integrin- $\beta 6$; using RT-PCR and immunocytochemistry, they confirmed that $\alpha\text{v}\beta 6$ integrin is restricted to sheep epithelial cells. The expression of this integrin was detected in epithelia of the airways, oral cavity, gastrointestinal tract, kidney, sweat glands, hair follicle sheaths, and the epidermis of the pedal coronary band, but not of normal skin. Consistent with the FMDV tropism, the researchers were able to detect the integrin $\alpha\text{v}\beta 6$ within the basal layers of the stratified squamous epithelium of the oral mucosa and coronary band. In addition, the authors show that the integrin $\alpha\text{v}\beta 6$ appears to be constitutively expressed in the normal airways of both cattle and sheep, and importantly, integrin $\alpha\text{v}\beta 6$ is expressed at high levels in the tonsillar crypt epithelium in sheep and by airway epithelia in both sheep and cattle, suggesting that ruminant airway epithelia represent the most likely portal of entry for the virus for initiation of infection with FMDV by inhalation.

It has been reported that the RGD motif in the G-H loop of the VP1 capsid protein of the FMDV forms a highly stable, EDTA-resistant complex with cellular inte-

grins; for $\alpha\beta 6$ this stable binding was shown to be dependent on a helical structure immediately C terminal to the RGD and, using peptides with specific amino acid substitutions, the authors demonstrated that two leucine residues at positions RGD +1 and RGD +4 are key points in forming the stable union. This feature indicates that the integrin-binding loop of FMDV has evolved to form a very stable complex with the receptor of the virus, and these highly stable complexes could contribute to the high infectivity of FMDV by increasing the proportion of attached virions and, therefore, the likelihood of virus internalization and infection [54].

On the other hand, a second approach to explore the $\alpha\beta 6$ integrin has been the use of a recombinant $\alpha\beta 6$ as a capture ligand in a sandwich ELISA for the detection and serotyping of FMDV. In this approach, the recombinant integrin bound FMDV of all seven known viral serotypes and the specificity of the reaction of the integrin capture/mAb detector combination was found to be superior to that of the more routinely employed rabbit/guinea pig ELISA. As a universal trapping reagent for all FMDV serotypes the $\alpha\beta 6$ recombinant protein also has the potential for application in other test procedures for viral identification and in antibody detection assays employed for the diagnosis of FMD [55].

Integrin $\alpha\beta 1$, a New Receptor for FMDV

Jackson et al. [56] showed that the integrin $\alpha\beta 1$, when expressed as a human/hamster heterodimer on transfected CHO2 cells, can act as a receptor for FMDV. Virus binding and infection mediated by $\alpha\beta 1$ was inefficient in the presence of physiological concentrations of calcium and magnesium; however, following treatment of $\alpha\beta 1$ expressing cells with either manganese ions or an activating anti- $\beta 1$ antibody, binding and infection by FMDV were dramatically enhanced [14, 56]. Moreover, on transfected CHO2 cells, virus binding was inhibited by nearly 100% by a function-blocking monoclonal antibody that specifically recognizes human $\alpha\beta$; and, finally, infection of transfected cells was also inhibited by >98% using the same antibody. In addition, RGD-containing peptides were shown to specifically inhibit virus attachment and infection mediated by $\alpha\beta 1$ [56].

Chimeric $\alpha 5/\alpha\beta$ integrin subunits in association with the $\beta 1$ chain have a ligand binding specificity identical to that of wild-type $\alpha\beta 1$. Studies of these chimeric integrins show that they are able to bind and mediate infection by FMDV, thus providing further evidence for the receptor role of $\alpha\beta 1$. In addition, further studies suggested that amino acid residues near the RGD motif may

be important for differentiating between the binding specificities of $\alpha\beta 1$ and $\alpha\beta 6$ [39, 48, 56].

As mentioned above, the efficiency of infection mediated by $\alpha\beta 1$ against three strains of FMDV serotype A and two strains of serotype O was evaluated in a transient expression assay system; the authors found that all viruses could infect cells expressing these integrin; however, type A viruses used $\alpha\beta 1$ with moderate efficiency compared with the $\alpha\beta 3$ and $\alpha\beta 6$ integrins. In contrast, both type O viruses used $\alpha\beta 1$ with same efficiency as $\alpha\beta 6$ integrins and with higher efficiency than $\alpha\beta 3$, implying a possible role for the efficiency of integrin usage in pathogenesis [47].

Integrin $\alpha\beta 8$ as a Receptor for FMDV

Jackson et al. [57] showed that a fourth $\alpha\beta$ integrin, $\alpha\beta 8$, can also function as a receptor for FMDV. Using SW480 cell line, which are normally nonpermissive for FMDV, the authors reported that it becomes susceptible to infection following transfection with human $\beta 8$ cDNA and expression of $\alpha\beta 8$ at the cell surface. The involvement of $\alpha\beta 8$ in infection was confirmed by showing that virus attachment to the transfected cells was inhibited by function-blocking monoclonal antibodies specific for either the $\alpha\beta 8$ heterodimer or the α .

Recently, $\alpha\beta 8$ has been identified in the basal cells of the epithelial airway (the major proliferative compartment of the proximal airway) in other mammalian species. If these observations were repeated in the natural host of FMDV, they would point to an important role for $\alpha\beta 8$ in the tropism and pathogenesis of FMDV during the initial phase of infection [57–59].

Glycosaminoglycans: Heparan Sulfate

Heparan sulfate, a glycosaminoglycan (GAG), is the most abundant heteropolysaccharide in the body; other common types of GAGs are chondroitin sulfate A, dermatan sulfate (chondroitin sulfate B), and keratan sulfate. GAGs are polymers of disaccharide repeats which are highly sulfated, and hence negatively charged, with an extended conformation that imparts high viscosity to the solution and they occur as the carbohydrate component of a family of glycoproteins known as proteoglycans. Proteoglycans are widely distributed in animal tissues, where they are found on virtually all cell types, both as parts of the extracellular matrix and as integral membrane components [60, 61].

The molecules of GAG are long unbranched polysaccharides containing a repeating disaccharide unit. The disaccharide units contain either of two modified sugars, N-acetylgalactosamine (GalNAc) or N-acetylglucosamine (GlcNAc) and an uronic acid such as glucuronate or iduronate. The high viscosity of GAGs is accompanied by low compressibility, making these molecules ideal as a lubricating fluid in joints. At the same time, their rigidity provides structural integrity to cells, helping to form passageways between cells and allowing for cell migration. The specific GAGs of physiological significance are hyaluronic acid, dermatan sulfate, chondroitin sulfate, heparin, heparan sulfate, and keratin sulfate. Although each of these GAGs has a predominant disaccharide component, heterogeneity exists in the sugar moieties of any given class of GAG [62].

The majority of GAGs in the body are linked to core proteins, forming proteoglycans (also called mucopolysaccharides). The GAGs extend perpendicularly from the core in a brush-like structure. Linkage of GAGs to the protein core involves a specific trisaccharide composed of two galactose residues and a xylose residue (GAG-Gal-GalXyl-O-CH₂ protein). The trisaccharide linker is coupled to the protein core via an O-glycosidic residue bound to a serine residue in the protein. The protein cores of proteoglycans are rich in serine and threonine residues that allow multiple GAG attachments [62].

Heparan Sulfate as an FMDV Receptor

In addition to the integrins, the second molecule involved as cellular receptors to FMDV is heparan sulfate. In 1996, Jackson et al. [61] reported that, in addition to an integrin-binding site, type O strains of FMDV share with natural ligands of $\alpha v \beta 3$ a specific affinity for heparan sulfate, the binding of which is the initial event in cellular entry and is required for efficient *in vitro* infection. Binding of the virus to paraformaldehyde-fixed cells was powerfully inhibited by agents such as heparin that compete with heparan sulfate, and by agents that compete for heparan sulfate such as platelet factor 4 or that inactivate it (heparinase). Neither chondroitin sulfate, a structurally related component of the extracellular matrix, nor dextran sulfate appreciably inhibited binding. These studies showed that the infection of cells by FMDV could also be blocked specifically by heparin, albeit at a higher concentration of inhibitor, caused a reduction in the number of plaques formed compared with that for untreated cells; and mutant cell lines deficient in heparan sulfate expression were unable to support plaque formation by FMDV, even though they remained equal-

ly susceptible to another picornavirus, bovine enterovirus, showing that entry of type O FMDV into cells is a complex process [61, 62].

The O1 type of FMDV binds GAGs through a shallow depression site on the virion surface, located at the junction of the three major capsid proteins, VP1, VP2 and VP3. Two preformed sulfate-binding sites control the receptor specificity and a residue of VP3, an arginine in this virus, is critical to this recognition, forming a key component of both sites. In field isolates of FMDV this residue is a histidine, switching to an arginine in adaptation to cell culture to form the high-affinity heparan sulfate-binding site. It was postulated that this site is a conserved feature of FMDVs, such that in the infected animal there is a biological advantage to low affinity, or more selective, interactions with GAG receptors [61, 63].

Sa-Carvalho et al. [64] in 1997 showed that *in vitro* cultivation of FMDV type O selects viruses that bind to heparin and that viruses with the heparin-binding phenotype are attenuated in the natural host. After this study, Baranowsky et al. [65] reported that FMDV-adapted tissue culture displayed enhanced affinity for heparin, but binding to cell surface heparan sulfate moieties was not required for expression of the hypervirulent phenotype of variants of FMDV serotype C in CHO cells.

The possibility that a weak binding to heparan sulfate serves to increase the rate of integrin-mediated virus uptake has been discussed. Alternatively, the site may be specific for some other, as yet unidentified, ligand in the natural host, presumably structurally related to heparan sulfate, and involved in cell entry [39]. It should also be considered that the potential to be a strong GAG binder is itself selectively advantageous, as was recently shown by O'Donnell et al. [66] in 2008, who analyzed the heparan sulfate binding and internalization of FMDV using a genetically engineered variant of O1Campos type that can use both integrins and heparan sulfate as receptors, and a second variant which can utilize only heparan sulfate as a receptor, and showed that heparan sulfate-binding FMDV enters the cells using the caveola-mediated endocytosis pathway and that caveolae can associate and traffic with endosomes.

Studies by Fry et al. [67], using crystallographic analysis evaluating the structure of a cell culture-adapted strain of FMDV called A1061 complexed with heparin, showed that this virus has an RGSD motif in place of the otherwise conserved RGD integrin-binding motif having also the potential to bind heparan sulfate. In this culture-adapted strain, the VP1 G-H loop comprising the integrin binding motif was disordered and heparin bound

at a similar site and in a similar conformation to that seen in the analogous complex with O1BFS, although the binding had a lower affinity and was more ionic.

FMDV Receptors Other than Heparan Sulfate and Integrins

Adaptation of FMDV to cell culture can result in changes in viral properties that include acquisition of the ability to bind to cell surface heparan sulfate instead of integrins. However, the adaptation of the Cathay topotype of FMDV to culture to produce vaccines has shown differences from the parental strain by a reduced ability to kill suckling mice, despite high infectivity in BHK cells. The evaluation of this phenomenon has shown that derivative viruses appear to replicate in cell culture in a manner that requires either binding to heparan sulfate or RGD-mediated binding to integrins, suggesting that multiple receptors and viral components can be utilized for mediating infection and disease by FMDV [68], and although this virus was tested in pigs, where it produced a mild disease, the epidemiological role of new viral variants still being unknown.

Concluding Remarks

FMDV is currently an important problem to both meat and milk producers. The understanding of the mechanism of infection and replication of this virus is important to the control of this worldwide threat. An important question that has yet to be addressed concerns the role of viral receptors in the pathogenesis of FMD. The aim of this review of FMDV receptors was to highlight the role of the specific receptor as a critical choice in determining the course of the disease in the infected animal, its transmission among animal populations in the field, and, taking into account that soluble receptors can interfere with FMDV infection *in vitro*, open a possible window to develop soluble receptors and anti-integrin compounds for use in FMD control strategies, as has been reported for a number of human pathologies resulting in the synthesis of small-molecule integrin antagonists and mimetics, which are active analogs of the protein ligands themselves, and could avoid the interaction between virus and receptors [69, 70].

Studies regarding integrin expression in the upper respiratory tract of the natural hosts of FMDV are limited, although today we know that $\alpha\text{v}\beta 6$ and multiple $\beta 1$ inte-

grins, but not $\alpha\text{v}\beta 3$, are expressed on mucosal epithelium, suggesting that $\alpha\text{v}\beta 6$ may have a prominent role in infection at these sites. Experiments using the bovine integrin $\alpha\text{v}\beta 3$ demonstrate that the use of bovine integrins increases adhesion and viral replication. *In vitro* studies using the chimeric human/hamster $\alpha\text{v}\beta 1$ and human $\alpha 5\beta 1$ tested their roles as cellular receptors in mediating infection by FMDV. Researchers showed that bovine $\alpha\text{v}\beta 1$ integrin could mediate FMDV infection; however, further studies are necessary to evaluate the expression and possible function *in vivo* of these receptors for FMDV. Little is known about the *in vivo* cell-type expression or tissue distribution of $\alpha\text{v}\beta 1$ since no complex specific antibodies are currently available. Therefore, the question of the role that $\alpha\text{v}\beta 1$ might play in *in vivo* infections with FMDV, if any, remains [56].

Unlike $\alpha\text{v}\beta 1$, $\alpha\text{v}\beta 3$, $\alpha 5\beta 11$ integrins, $\alpha\text{v}\beta 6$ integrin did not require activation by divalent cations such as Mn, Mg and Ca; despite an increase in the concentrations of these cations, the cellular expression of $\alpha\text{v}\beta 6$ was not increased. Taking into account the aforementioned conclusions, plus the fact that $\alpha\text{v}\beta 6$ is constitutively expressed in the normal airways of cattle, the studies with soluble integrins, showing that soluble $\alpha\text{v}\beta 6$ can interfere with FMDV infection *in vitro*, indicate $\alpha\text{v}\beta 6$ blockade as a potential control point in the FMDV lifecycle. The development of therapeutic tools using this integrin as target may enable the generation of a possible strategy to control FMDV infection *in vivo* or at least help to control virus spread during an outbreak. Also as was reported and mentioned before, the fact that the $\alpha\text{v}\beta 6$ integrin can recognize all known FMDV serotypes makes it pretty advantageous for development of new and more specific diagnostic tools that could be internationally used, as was shown by Ferris et al. [55] who used it as a capture ligand in a sandwich ELISA, showing a high potential for using the recombinant integrin in FMDV test procedures as a universal trapping reagent for all viral serotypes.

In vitro heparan sulfate is a good receptor for FMDV. Previously described studies demonstrated that when heparan sulfate is expressed together with integrins, the virus has greater affinity by the heparan sulfate. However, the role *in vivo* of this GAG in FMDV infection is unknown. The selective pressure on field strains to adapt to cell culture may be an item of practical concern to the vaccine industry, due to the selectivity of field isolates in their choice of integrin which grow very poorly in cell culture in the absence of expression of their required integrin.

As has been discussed by O'Donnell et al. [66], although it has been demonstrated that heparan sulfate-binding FMDV are relatively avirulent in vivo [64], it is important to evaluate if FMDV can infect cells using al-

ternative unknown mechanisms due to the field variability of FMDV and the emergence of new viral strains, which might manifest different and/or new ways of viral entry.

References

- Manson PW, Grubman MJ, Baxt B: Molecular basis of pathogenesis of FMDV. *Virus Res* 2003;91:9–32.
- Domingo E, Escarmís C, Baranowski E, Ruiz-Jarabo CM, Carrillo E, Núñez JI, Sobrino F: Evolution of foot and mouth disease virus. *Virus Res* 2003;91:47–63.
- Knowles NJ, Samuel AR: Molecular epidemiology of foot and mouth disease virus. *Virus Res* 2003;91:65–80.
- Jackson T, King AM, Stuart D, Fry E: Structure and receptor binding. *Virus Res* 2003;91:33–46.
- Burman A, Clark S, Abrescia NG, Fry EE, Stuart DI, Jackson T: Specificity of the VP1 GH loop of foot-and-mouth disease virus for alpha-v integrins. *J Virol* 2006;80:9798–9810.
- Fry EE, Stuart DI, Rowlands DJ: The structure of foot-and-mouth disease virus. *Curr Top Microbiol Immunol* 2005;288:71–101.
- Alcalá P, Feliu JX, Arís A, Villaverde A: Efficient accommodation of recombinant, foot-and-mouth disease virus RGD peptides to cell-surface integrins. *Biochem Biophys Res Commun* 2001;285:201–206.
- Alexandersen S, Zhang Z, Donaldson AI, Garland AJ: The pathogenesis and diagnosis of foot-and-mouth disease. *J Comp Pathol* 2003;129:1–36.
- Thomson GR, Vosloo W, Bastos AD: Foot and mouth disease in wildlife. *Virus Res* 2003;91:145–161.
- Grubman MJ, Baxt B: Foot-and-mouth disease. *Clin Microbiol Rev* 2004;17:465–493.
- Doel TR: FMD vaccines. *Virus Res* 2003;91:81–99.
- OIE: World Organization for Animal Health 2006: Foot And Mouth Disease Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Mammals, Birds and Bees), ed 5. Paris, World Organization for Animal Health, 2006, vol 1, 2, chap 2.1.1.
- O'Donnell V, LaRocco M, Duque H, Baxt B: Analysis of foot-and-mouth disease virus internalization events in cultured cells. *J Virol* 2005;79:8506–8518.
- Berryman S, Clark S, Monaghan P, Jackson T: Early events in integrin $\alpha\beta 6$ mediated cell entry of foot-and-mouth disease virus. *J Virol* 2005;79:8519–8534.
- Monaghan P, Simpson J, Murphy C, Durand S, Quan M, Alexandersen S: Use of confocal immunofluorescence microscopy to localize viral nonstructural proteins and potential sites of replication in pigs experimentally infected with foot-and-mouth disease virus. *J Virol* 2005;79:6410–6418.
- Springer TA: Predicted and experimental structures of integrins and β -propellers. *Curr Opin Struct Biol* 2002;12:802–813.
- Miller LC, Blakemore W, Sheppard D, Atakilit A, King AM, Jackson T: Role of the cytoplasmic domain of the b-subunit of integrin $\alpha\beta 6$ in infection by foot-and-mouth disease virus. *J Virol* 2001;75:4158–4164.
- Grundstrom G: Functional studies of collagen-binding integrins $\alpha 2\beta 1$ and $\alpha 1\beta 1$. Interplay between integrins and platelet-derived growth factor receptors. *Acta Universitatis Upsaliensis. Comprehensive summaries of Uppsala Dissertations from Faculty of Medicine, Uppsala University*, 2003, p 97.
- Hynes RO: Integrins: versatility, modulation, and signaling in cell adhesion. *Cell* 1992;69:11–25.
- Hynes RO: Integrins: bidirectional, allosteric signaling machines. *Cell* 2002;100:673–687.
- Dedhar S, Hannigan GE: Integrin cytoplasmic interactions and bidirectional transmembrane signaling. *Curr Opin Cell Biol* 1996;8:657–669.
- Montgomery AM, Reisfeld RA, Cheresh DA: Integrin $\alpha\beta 3$ rescues melanoma cells from apoptosis in three dimensional dermal collagen. *Proc Natl Acad Sci USA* 1994;91:8856–8860.
- O'Toole TE, Katagiri Y, Faull RJ, Peter RK, Tamura R, Quaranta R, Loftus JC, Shattil SJ, Ginsberg MH: Integrin cytoplasmic domains mediate inside-out signal-transduction. *J Cell Biol* 1994;124:1047–1059.
- Faull RJ, Ginsberg MH: Inside-out signaling in integrins. *Education Program 26th Congr Int Soc Haematology, Singapore*, 1996.
- Hughes PE, Pfaff M: Integrin affinity modulation. *Trends Cell Biol* 1998;8:358–364.
- Plow EF, Haas TA, Zhang L, Loftus J, Smith JW: Ligand binding to integrins. *J Biol Chem* 2000;275:21785–21788.
- Dickenson SK, Mathis NL, Rahman M, Bergelson JM, Santoro SA: Determinants of ligand binding specificity of the alpha(1) beta(1) and alpha(2) beta(1) integrins. *J Biol Chem* 1999;274:32182–32191.
- Hemler ME: Integrin associated proteins. *Curr Opin Cell Biol* 1998;10:578–585.
- Humphries MJ: Integrin structure. *Biochem Soc Trans* 2000;28:311–339.
- Mould AP, Akiyama SK, Humphries MJ: Regulation of integrin $\alpha\beta 1$ -fibronectin interactions by divalent cations. *J Biol Chem* 1995;270:26270–26277.
- Smith JW, Piotrowicz RS, Mathis D: A mechanism for divalent cation regulation of $\beta 3$ -integrins. *J Biol Chem* 1994;269:960–967.
- Hu DD, Hoyer JR, Smith JW: Ca21 suppresses cell adhesion to osteopontin by attenuating binding affinity for integrin $\alpha\beta 3$. *J Biol Chem* 1995;270:9917–9925.
- Mateu MG, Valero ML, Andreu D, Domingo E: Systematic replacement of amino acid residues within an Arg-Gly-Asp-containing loop of foot-and-mouth disease virus and effects on cell recognition. *J Biol Chem* 1996;271:12814–12819.
- Xiong JP, Stehle T, Zhang R, Joachimiak A, Frech M, Goodman SL, Arnaout MA: Crystal structure of the extracellular segment of integrin $\alpha\beta 3$ in complex with an Arg-Gly-Asp ligand. *Science* 2002;296:151–155.
- Berinstein A, Roivainen M, Hovi T, Mason PW, Baxt B: Antibodies to the vitronectin receptor (integrin $\alpha\beta 3$) inhibit binding and infection of foot-and-mouth disease virus to cultured cells. *J Virol* 1995;69:2664–2666.
- Mason PW, Reider E, Baxt B: RGD sequence of foot-and mouth disease virus is essential for infecting cells via the natural receptor but can be bypassed by an antibody-dependant enhancement pathway. *Proc Natl Acad Sci USA* 1994;91:1932–1936.
- Leippert M, Beck E, Weiland F, Pfaff E: Point mutations within the bG-bH loop of foot-and-mouth disease virus O1K affects attachment to target cells. *J Virol* 1997;71:1046–1051.
- Neff S, Mason PW, Baxt B: High-efficiency utilization of the bovine integrin $\alpha\beta 3$ as a receptor for foot-and-mouth disease virus is dependent on the bovine $\beta 3$ subunit. *J Virol* 2000;74:7298–7306.
- Neff S, Sa-Carvalho D, Rieder E, Mason PW, Blystone SD, Brown EJ, Baxt B: Foot-and-mouth disease virus virulent for cattle utilizes the integrin $\alpha\beta 3$ as its receptor. *J Virol* 1998;72:3587–3594.

- 40 Calvete JJ, Henschen A, González-Rodríguez J: Assignment of disulphide bonds in human platelet GPIIb: a disulphide pattern for the b-subunits of the integrin family. *Biochem J* 1991;274:63–71.
- 41 Green L, Mould AP, Humphries MJ: The integrin beta subunit. *Int J Biochem Cell Biol* 1998;30:179–184.
- 42 Jackson T, Sharma A, Ghazaleh RA, Blakemore WE, Ellard FM, Simmons DL, Newman JW, Stuart DI, King AM: Arginine-glycine-aspartic acid-specific binding by foot-and-mouth disease viruses to the purified integrin $\alpha\text{v}\beta 3$ in vitro. *J Virol* 1997;71:8357–8361.
- 43 Neff S, Baxt B: The ability of integrin $\alpha\text{v}\beta 3$ to function as a receptor for foot-and-mouth disease virus is not dependent on the presence of complete subunit cytoplasmic domains. *J Virol* 2001;75:527–532.
- 44 Monaghan P, Gold S, Simpsom J, Zhang Z, Weinreb PH, Violette SM, Alexandersen S, Jackson T: The $\alpha\text{v}\beta 6$ integrin receptor for foot-and-mouth disease virus is expressed constitutively on the epithelial cells targeted in cattle. *J Gen Virol* 2005;86:2769–2780.
- 45 Jackson T, Blakemore W, Newman JW, Knowles NJ, Mould AP, Humphries MJ, King AM: Foot-and-mouth disease virus is a ligand for the high-affinity binding conformation of integrin $\alpha 5\beta 1$: influence of the leucine residue within the RGD motif on selectivity of integrin binding. *J Gen Virol* 2000;81:1383–1391.
- 46 Baranowski E, Ruiz-Jarabo CM, Sevilla N, Andreu D, Beck E, Domingo E: Cell recognition by foot-and-mouth disease virus that lacks the RGD integrin-binding motif: flexibility in *Aphthovirus* receptor usage. *J Virol* 2000;74:1641–1647.
- 47 Duque H, Baxt B: Foot-and-mouth disease virus receptors: comparison of bovine alpha(V) integrin utilization by type A and O viruses. *J Virol* 2003;77:2500–2511.
- 48 Jackson T, Sheppard D, Denyer M, Blakemore W, King AM: The epithelial integrin $\alpha\text{v}\beta 6$ is a receptor for foot-and-mouth disease virus. *J Virol* 2000b;74:4949–4956.
- 49 O'Donnell V, LaRocco M, Duque H, Baxt B: Analysis of foot-and-mouth disease virus internalization events in cultured cells. *J Virol* 2005;79:8506–8518.
- 50 Rieder E, Henry T, Duque H, Baxt B: Analysis of a foot-and-mouth disease virus type A24 isolate containing an SGD receptor recognition site in vitro and its pathogenesis in cattle. *J Virol* 2005;79:12989–12998.
- 51 Breuss JM, Gillett N, Lu L, Sheppard D, Pytela R: Restricted distribution of integrin $\beta 6$ mRNA in primate epithelial tissues. *J Histochem Cytochem* 1993;41:1521–1527.
- 52 Duque H, LaRocco M, Golde Wt, Baxt B: Interactions of foot-and-mouth disease virus with soluble bovine $\alpha\text{v}\beta 3$ and $\alpha\text{v}\beta 6$ integrins. *J Virol* 2004;78:9773–9781.
- 53 Brown JK, McAleese SM, Thornton EM, Pate JA, Schock A, Macrae AI, Scott PR, Miller HRP, Collie DD: Integrin- $\alpha\text{v}\beta 6$, a putative receptor for foot-and-mouth disease virus, is constitutively expressed in ruminant airways. *J Histochem Cytochem* 2006;54:807–816.
- 54 Dicara D, Burman A, Clark S, Berryman S, Howard MJ, Hart IR, Marshall JF, Jackson T: Foot-and-mouth disease virus forms a highly stable, EDTA-resistant complex with its principal receptor, integrin $\alpha\text{v}\beta 6$: implications for infectiousness. *J Virol* 2008;82:1537–1546.
- 55 Ferris NP, Abrescia NG, Stuart DI, Jackson T, Burman A, King DP, Paton DJ: Utility of recombinant integrin $\alpha\text{v}\beta 6$ as a capture reagent in immunoassays for the diagnosis of foot-and-mouth disease. *J Virol Method* 2005;127:69–79.
- 56 Jackson T, Mould AP, Sheppard D, King AM: Integrin $\alpha\text{v}\beta 1$ is a receptor for foot-and-mouth disease virus. *J Virol* 2002;76:935–941.
- 57 Jackson T, Clark S, Berryman S, Burman A, Cambier S, Mu D, Nishimura S, King A: Integrin $\alpha\text{v}\beta 8$ functions as a receptor for foot-and-mouth disease virus: role of the β -chain cytodomain in integrin-mediated infection. *J Virol* 2004;78:4533–4540.
- 58 Cambier S, Mu D, O'Connell D, Boylen K, Travis W, Liu W, Broadus VC, Nishimura SL: A role for the integrin $\alpha\text{v}\beta 8$ in the negative regulation of epithelial cell growth. *Can Res* 2000;60:7084–7093.
- 59 Fjellbirkeland L, Cambier S, Broadus VC, Hill A, Brunetta P, Dolganov G, Jablons D, Nishimura SL: Integrin $\alpha\text{v}\beta 8$ -mediated activation of transforming growth factor α inhibits human airway epithelial proliferation in intact bronchial tissue. *Am J Pathol* 2003;163:533–542.
- 60 Kjellen L, Lindah U: Proteoglycans: structures and interactions. *Annu Rev Biochem* 1991;60:443–475.
- 61 Jackson T, Ellard FM, Ghazaleh RA, Brookes SM, Blakemore WE, Corteyn AH, Stuart DI, Newman JW, King AM: Efficient infection of cells in culture by type O foot-and-mouth disease virus requires binding to cell surface heparan sulfate. *J Virol* 1996;70:5282–5287.
- 62 Haywood AM: Virus receptors: binding, adhesion strengthening, and changes in viral structure. *J Virol* 1994;68:1–5.
- 63 Fry EE, Lea SM, Jackson T, Newman JW, Ellard FM, Blakemore WE, Ghazaleh RA, Samuel A, King AM, Stuart DI: The structure and function of a foot-and-mouth disease virus-oligosaccharide receptor complex. *EMBO J* 1999;18:543–554.
- 64 Sa-Carvalho D, Rieder E, Baxt B, Rodarte R, Tanuri A, Mason PW: Tissue culture adaptation of foot-and-mouth disease virus selects viruses that bind to heparin and are attenuated in cattle. *J Virol* 1997;71:5115–5123.
- 65 Baranowsky E, Sevilla N, Verdaguier N, Ruiz-Jarabo CM, Beck E, Domingo E: Multiple virulence determinants of foot-and-mouth diseases virus in cell culture. *J Virol* 1998;72:6362–6372.
- 66 O'Donnell V, Larocco M, Baxt B: Heparan sulfate-binding foot-and-mouth disease virus enters cells via caveola-mediated endocytosis. *J Virol* 2008;82:9075–9085.
- 67 Fry EE, Newman JW, Curry S, Najjam S, Jackson T, Blakemore W, Lea SM, Miller L, Burman A, King AM, Stuart DI: The structure of foot-and-mouth disease virus serotype A1061 alone and complexed with oligosaccharide receptor: receptor conservation in the face of antigenic variation. *J Gen Virol* 2005;86:1909–1920.
- 68 Zhao Q, Pacheco JM, Mason PW: Evaluation of genetically engineered derivatives of a Chinese strain of foot-and-mouth disease virus reveals a novel cell-binding site which functions in cell culture and in animals. *J Virol* 2003;77:3269–3280.
- 69 Gadek TR, McDowell RS: Discovery of small molecule leads in a biotechnology data-stream. *Drug Discov Today* 2003;8:545–550.
- 70 Shimaoka M, Springer TA: Therapeutic antagonists and conformational regulation of integrin function. *Nat Rev Drug Discov* 2003;2:703–716.