TURUN YLIOPISTON JULKAISUJA
ANNALES UNIVERSITATIS TURKUENSIS

SARJA – SER. D OSA– TOM. 908

MEDICA - ODONTOLOGICA

THE ROLE OF INTEGRINS IN ENTEROVIRUS INFECTIONS AND IN METASTASIS OF CANCER

by

Åse Karttunen

TURUN YLIOPISTO
UNIVERSITY OF TURKU
Turku 2010

TURUN YLIOPISTON JULKAISUJA
ANNALES UNIVERSITATIS TURKUENSIS

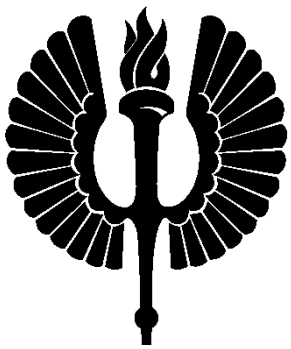
SARJA – SER. D OSA– TOM. 908

MEDICA - ODONTOLOGICA

THE ROLE OF INTEGRINS IN ENTEROVIRUS INFECTIONS AND IN METASTASIS OF CANCER

by

Åse Karttunen



TURUN YLIOPISTO
UNIVERSITY OF TURKU
Turku 2010

From the Department of Virology, University of Turku, Turku, the Department of Virology, Haartman Institute, the Helsinki Biomedical Graduate School, University of Helsinki, Helsinki, and the Department of Biochemistry and Pharmacy, Åbo Akademi University, Turku, Finland.

Supervised by

Professor Timo Hyypiä
Department of Virology
University of Turku
Turku, Finland

Reviewed by

Professor Klaus Hedman
Haartman Institute
Department of Virology
University of Helsinki
Helsinki, Finland

and

Docent Arno Hänninen
Department of Medical Microbiology and Immunology
University of Turku
Turku, Finland

Opponent

Professori Ari Hinkkanen
A. I. Virtanen-instituutti
Bioteknologia ja molekulaarinen lääketiede
Itä-Suomen yliopisto
Kuopio, Finland

ISBN 978-951-29-4313-5 (PRINT)
ISBN 978-951-29-4314-2 (PDF)
ISSN 03559483
Helsinki University Printing House
Helsinki 2010

To my Family

ABSTRACT

Åse Karttunen

THE ROLE OF INTEGRINS IN ENTEROVIRUS INFECTIONS AND IN METASTASIS OF CANCER

The Department of Virology, University of Turku, Turku, the Department of Virology, Haartman Institute, and the Helsinki Biomedical Graduate School, University of Helsinki, Helsinki, and the Department of Biochemistry and Pharmacy, Åbo Akademi University, Turku, Finland. Annales Universitatis Turkuensis, Medica-Odontologica, Yliopistopaino, Helsinki, 2010.

Integrins are a family of transmembrane glycoproteins, composed of two different subunits (α and β). Altered expression of integrins in tumor cells contributes to metastasis tendency by influencing on the cells' attachment to adjacent cells and their migration. Viral pathogens, including certain enteroviruses, use integrins as receptors. Enteroviruses have also been suggested to be involved in the etiopathogenesis of type 1 diabetes.

The study focuses on the role of integrins in the pathogenesis of metastasis to cortical bone and on type 1 diabetes (T1D) and echovirus 1 infection. In **the first part** of the thesis, the role of different integrins in the initial attachment of MDA-MD-231 breast cancer cells to bovine cortical bone disks was studied. A close correlation between α2β1 and α3β1 integrin receptor expression and the capability of the tumor to attach to bone were observed. In **the second part,** a possible correlation between susceptibility to enterovirus infections in diabetic children and differences in enterovirus receptor genes, including certain integrins, was investigated. In parallel, virus-specific neutralizing antibodies and diabetic risk alleles were studied. In the diabetic group, an amino acid change was detected in the polio virus receptor and the neutralizing antibody titers against echovirus 30 were lower. However, to obtain statistically sustainable results, a larger number of individuals should be analyzed. Echovirus 1 (EV1) enters cells by attaching to the α2I domain of the α2β1 integrin. In **the third part** EV1 was shown to attach to a chimeric receptor construct of the transferrin receptor and the α2I domain and to enter cells through clathrin-mediated endocytosis that is normally not used by the virus. The chimeric receptor was recycled to the plasma membrane, whereas the virus remained in intracellular vesicles. The virus replication cycle was initiated in these cells, suggesting that evolution pressure could possibly cause the virus to evolve to use a different entry mechanism. Moreover, a cDNA microarray analysis of host gene expression during EV1 replication showed that 0.53% of the total genes, including several immediate early genes, were differently expressed.

Keywords: Integrin, breast cancer, metastasis, human enterovirus, echovirus 1, type 1 diabetes, clathrin mediated endocytosis

TIIVISTELMÄ

Åse Karttunen

INTEGRIINIEN MERKITYS ENTEROVIRUSINFEKTIOSSA JA SYÖVÄN ETÄPESÄKKEIDEN MUODOSTUMISESSA

Virusoppi, Turun Yliopisto, Haartman-instituutti, Virologian osasto ja Biolääketieteellinen tutkijakoulu, Helsingin Yliopisto, Helsinki, ja Biokemian ja farmasian laitos, Åbo Akademi, Turku. Annales Universitatis Turkuensis, Yliopistopaino, Helsinki, 2010.

Integriinit ovat solukalvon läpäiseviä glykoproteiineja, jotka koostuvat kahdesta alayksiköstä (alfa ja beta). Ne ovat vuorovaikutuksessa solukalvon kanssa soluadheesiossa ja migraatiossa. Muuttunut integriinien ilmentyminen syöpäsoluissa vaikuttaa soluadheesioon ja soluliikkuvuuteen ja voi täten vaikuttaa solun taipumukseen muodostaa etäpesäkkeitä. Tiettyjen integriinien tuotto rintasyövässä korreloituu sairauden ennusteeseen ja metastasointiherkkyyteen. Eräät enterovirukset hyödyntävät solun integriinejä päästääkseen solun sisälle. Enterovirusten aiheuttamat taudinkuvat ihmisissä vaihtelevat nuhakuumeista sydänlihastulehduksiin ja keskushermostoinfektioihin. Enterovirusinfektioilla voi olla merkitystä myös tyypin I diabeteksen puhkeamisessa.

Tässä väitöskirjatyössä on tutkittu integriinien osuutta syövän etäpesäkkeiden leviämisessä luukudokseen sekä niiden toimintaa enterovirusten, kuten echovirus 1:n reseptoreina ja reseptorien ilmentymistä suhteessa tyypin I diabetekseen. Väitöskirjan **ensimmäisessä osatyössä** tutkittiin integriinien merkitystä rintasyöpäsolujen varhaisessa kiinnittymisessä kortikaalisille luukiekoille. Vahva yhteys nähtiin α2β1 ja α3β1 integriinien ilmentymisessä ja kyvyssä sitoutua kortikaaliseen luukudokseen. **Toisessa osatyössä** tutkittiin, voisivatko muutokset enterovirusten reseptorimolekyyleissä korreloida tyypin 1 diabeteksen puhkeamiseen. Sekvenssianalyysi tehtiin kuuden tunnetuimman enterovirusreseptorin (PVR, ICAM-1, DAF, CAR, αvβ3 ja α2β1 integriini) geenialueilta, ja virusspesifiset vasta-aineet määritettiin seerumista. Diabetesryhmästä geenivaihtelua löytyi PVR:sta ja vasta-aineet echovirus 30:tä vastaan olivat matalammat kuin kontrolliryhmässä. Tulokset pitää kuitenkin vielä varmistaa suuremmassa ryhmässä tilastollisen luotettavuuden selvittämiseksi. Echovirus I (EV1) sitoutuu α2β1 integriinin α2 alayksikön I-domeeniin (α2I), ja **kolmannessa osatyössä** osoitettiin että virus pystyi infektoimaan solut, jotka ilmensivät pinnallaan reseptorikimeeraa, missä α2I-jakso oli liitetty transferriinireseptoriin. Virus kuljetettiin soluun klatriinivälitteisesti, mikä eroaa EV1:n normaalisti käyttämistä soluuntukeutumisreiteistä. Lisäksi cDNA-mikrosiruanalyysi isäntäsolun geenienekspressiosta EV1-replikaation aikana osoitti, että 0.53% geenienekspressiosta oli muuttunut, sisältäen monia solun toiminnalle keskeisiä geenejä.

CONTENTS

ABBREVIATIONS

Ad	Adenovirus
α2I	I domain of the integrin α2 subunit
ALS	amyotrophic lateral sclerosis
Arf	ADP-ribosylation factor
ATF	activating transcription factor
β2-m	β2 microglobulin
BSA	bovine serum albumin
CAMs	cell adhesion molecules
CAR	coxsackievirus-adenovirus receptor
CAV	coxsackievirus A
CBV	coxsackievirus B
CD155	poliovirus and vitronectin receptor
Cdk	cyclin-dependent kinase
cDNA	complementary DNA
CLIC	clathrin-independent carrier
CPE	cytopathic effect
CI-MPR	mannose 6-phosphate receptor
CPV	canine parvovirus
CTGF	connective tissue growth factor
CXCL10	CXC chemokine ligand 10
DAF	decay-accelerating factor
ECM	extracellular matrix
ECV	endosomal carrier vesicle
EE	early endosome
EEA1	early endosome antigen 1
eIF4F	eucaryotic initiation factor 4F
EGF	epidermal growth factor
EGR1	early growth response 1
ER	endoplasmic reticulum
ERC	endocytic recycling compartment
EV	echovirus
FACS	flow cytometry (fluorescence activated cell sorting)
FAK	focal adhesion kinase
FasL	fas ligand
FPV	feline panleukopenia virus
Fra-1	fos related antigen-1
GAD65	glutamic acid decarboxylase 65
GAP	GTPase activating protein
GDI	rho dissociate inhibitor
GEEC	GPI-anchored, protein-enriched early endosomal compartment
GEFs	guanine nucleotide exhange factors

GPI	glycosylphosphatidylinositol
GRP78	glucose-regulated protein 78
HAVcr-1	HAV cellular receptor 1
HBV	hepatitis B virus
FMDV	foot-and-mouth-disease
HEV	human enteroviruses
HIV-1	human immunodeficiency virus type I
HPEV	human parechovirus
HRV	human rhinovirus
HS	heparan sulphate
HSV	herpes simplex virus
ICAM-1	intercellular adhesion molecule 1
IEG	immediate early gene
IF	immunofluorescence
IFN	interferon
IgSF	immunoglobulin superfamily
IRES	internal ribosome entry site
JNK	c-Jun NH2-terminal kinase
LDLR	low density lipoprotein receptor
LE	late endosome
LFA-1	lymphocyte function associated antigen-1
VLDLR	very low density lipoprotein receptor
LRP1	LDLR related protein 1
MAb	monoclonal antibody
MAPK	p38 mitogen-activated protein kinase
MIDAS	metal-ion-dependent adhesion site
MMP	matrix metalloproteinase
MOI	multiplicity of infection
OPN	osteopontin
OPV	oral polio vaccine
PABP	poly(A)-binding protein
PARP	poly(ADP-ribose) polymerase
Pak	p21-activated kinase
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PI3K	phosphoinositide 3-kinase
PI(3)P	phospatidylinositol 3-kinase
PLC	phospholipase C
PBMC	peripheral blood mononuclear cell
pol II	RNA polymerase II
PSI	plexin-semaphorin-integrin
PV	poliovirus
PVR	poliovirus and vironectin receptor
RabGDI	Rab GDP dissociation inhibitor

REP	Rab escort protein
RGD	arginine-glycine-aspartic acid
SDS	sodium dodecyl sulfate
SFV	Semliki forest virus
SNP	single nucleotide polymorphism
SRC	short consensus repeats
SV40	simian virus 40
T1D	type 1 diabetes
Tf	transferrin
TF	transcription factor
TfR	transferrin receptor
TJ	tight junction
TM	transmembrane
TNF	tumor necrosis factor
u-PAR	urokinase-plasminogen activator receptor
UTR	untranslated region
VCAM-1	vascular cell adhesion molecule-1
VLDL-R	very-low-density lipoprotein receptor
vp1	enteroviral capsid protein vp1
VWA	von Willebrand A domains
vRNA	viral RNA

LIST OF ORGINAL PUBLICATIONS

This thesis is based on the following orginal publications, which are referred to in the text by Roman numerals (I-III). In addition, results about cellular gene expression during echovirus 1 infection are reported.

I Lundström, Å., Holmbom, J., Lindqvist, C. & **Nordström, T.** 1998. The role of $\alpha 2\beta 1$ and $\alpha 3\beta 1$ integrin receptors in initial anchoring of MDA-MB-231 human breast cancer cells to cortical bone matrix. Biochemical and Biophysical Research Communications 250:735-40.

II Karttunen, Å., Pöyry, T., Vaarala, O., Ilonen, J., Hovi, T., Roivainen, M. & Hyypiä, T. 2003. Variation in enterovirus receptor genes. Journal of Medical Virology 70:99-108.

III Karttunen, Å., Pietiäinen, V., Pukkila, **V., Heino, J., Waris, M., Marjomäki, V., & Hyypiä, T.** Targeting of echovirus 1 to the clathrin pathway. Submitted

The original papers have been reprinted with the kind permission of the copyright holders.

1 INTRODUCTION

Integrins are a family of transmembrane glycoproteins that interact with the extracellular matrices in cell adhesion and migration. Altered expression of integrins in tumor cells contribute to metastasis tendency by affecting attachment to adjacent cells and migration tendency. Breast cancer is characterized by frequent skeletal metastasis. One possibility that might explain the bone selectivity is that the breast cancer cells express surface adhesion molecules that facilitate their adherence to proteins present in the bone microvasculature matrix. In addition to natural ligands (such as vitronectin and collagen), viral pathogenes, including certain enteroviruses, utilize the cellular integrin receptors to enter the host cells. Enteroviruses belonging to the picornavirus group are among the most common viruses infecting humans globally. Several studies have suggested that enteroviruses could also be involved in the etiopathogenesis of type 1 diabetes.

This thesis aims to elucidate the role of enterovirus receptors including the integrins in the pathogenesis of metastasis to cortical bone and type 1 diabetes and echovirus 1 infection in four different subprojects. In the first subproject, the most important integrins in initial anchorage of human breast cancer to cortical bone matrix were investigated. In the second subproject, a possible correlation between the susceptibility to enterovirus infections and/or frequent occurrence of enterovirus antibodies in prediabetic and diabetic children with differences in integrin and other enterovirus receptor genes were studied. In parallel, virus-specific neutralizing antibodies and diabetic risk alleles were investigated. The third and fourth subproject concentrated on echovirus 1 (EV1) which uses α2β1 integrin as a cellular receptor. In the third subproject, the EV1 entry via a chimeric receptor construct of transferrin receptor and α2 I domain was studied. The aim was to analyze if EV1 is able enter cells by clathrin directed entry which is normally not used by the virus, and establish productive infection. In the fourth subproject the host gene expression was investigated during EV1 endocytosis and replication.

2 REVIEW OF THE LITERATURE

2.1 INTEGRINS

Integrins are a family of cell surface glycoproteins composed of at least 18 different α subunits and eight β subunits that attach noncovalently forming 24 different heterodimers (Hynes, 2002) (Fig 1., Table 1). The extracellular N-domain of the α- and β-subunits have up to 1104 residues and 778 residues respectively, a single transmembrane (TM) domain and a C-terminal, cytoplasmic tail. Integrins function as receptors for extracellular matrix (ECM) proteins and are important in cell-cell interactions during differentiation, malignant transformation, immune recognition and blood coagulation. Integrins bind to diverse ligands, including many ECM proteins such as collagen, fibronectin, laminin, vitronectin, von Willebrand factor and thrombospondin, ICAMs, members of the immunoglobulin superfamily, (IgSF) and plasma proteins, like fibrinogen (Hynes & Lander, 1992). In addition, a restricted number of proteins interact with integrins within the plasma membrane bilayer. These include caveolins, leukocyte surface antigen CD47, urokinase-plasminogen activator receptor (u-PAR) and certain tetraspanins (Barberis *et al*., 2000, Chapman *et al*., 1999, Giancotti, 2000, Hemler *et al*., 1985).

Nine of the vertebrate α-subunits contain an approximately 190 amino acids long I (”inserted’’) domain (Whittaker & Hynes, 2002). In those I domain integrins, the domain is the major ligand binding site. The I domain is a member of a family of von Willebrand A domains (VWA). The globular domain is composed of a GTPase-like domain, with a metal-ion-binding site (MIDAS). Dependent of the conformation of MIDAS, I domains can exist in both high affinity and low affinity ligand-binding conformation (Emsley *et al*., 2000, Lee *et al*., 1995a, Lee *et al*., 1995b) that is essential for binding of some ligands, e.g. collagen (Emsley *et al*., 2000). The I domain interacts with a β-propeller, which is a G-protein-like domain formed from seven similar structural units (Springer, 1997). In integrins lacking a αI domain, the main ligand-binding sites are in the βI domain, the β-propeller and the upper surface of the α subunit (Xiong *et al*., 2001).

The leg of the α-subunit is composed of a thigh domain and two calf domains. All β-subunits contain a head with an I-like domain, which shares common structures with the αI domains (Lee *et al*., 1995a) including MIDAS. The leg of the β-subunit consists of a plexin-semaphorin-integrin (PSI) domain, hybrid domain, four cystein–rich repeats (I-EGF; epidermal growth factor domains) and a novel cystatin-like fold (Hynes, 2002).
The cytoplasmic tail is crucial for modulating ligand-integrin interactions into dynamic cellular responses, such as cell spreading or migration and endocytosis. The intracellular

Table 1. Human Integrins (Modified from Johnson et al., 2009)

Integrin	Ligands
Integrins recognize the RGD peptide	
α5β1	Fibronectin
α8β1	Fibronectin, vitronectin, tenacin C, osteopontin, nefronectin
αVβ4	Fibronectin, vitronectin
αVβ3	Fibrinogen, fibronectin, vitronectin, tenascin C, osteopontin, bone sialoprotein, MMP-2, CYR61
αVβ5	Vitronectin
αVβ6	Fibronectin, TGF-β-LAP
αVβ8	Vitronectin
αIIbβ3	Fibrinogen, fibronectin, vitronectin
The Integrin Collagen receptor subfamily	
α1β1	Collagens, semaphorin 7A (laminins)
α2β1	Collagens, tenascin C, (laminins)
α10β1	Collagens
α11β1	Collagens
Leucocyte integrin subfamily	
αDβ2	ICAM, VCAM
αMβ2	ICAM, VCAM, iC3b, factor X, fibrinogen
αLβ2	ICAM,
αXβ2	Fibrinogen, plasminogen, heparin, iC3b
α4β7	Fibronectin, VCAM
αEβ7	E-cadherin
Integrins containing an α3, α4, α6, α7 or α9 α subunit	
α3β1	Laminins (collagens)
α4β1	Fibronectin, VCAM
α6β1	Laminins
α6β4	Laminins
α7β1	Laminins
α9β1	Tenascin C, osteopontin, ADAMs, factor XIII, VCAM, VEGF-C, VEGF-D

domains are linked to the actin cytoskeleton and several adapter molecules including vinculin, talin or α-actin (Zamir & Geiger, 2001). All integrins, except α6β4, are coupled to the actin cytoskeleton. The β4 integrin subunit differs from the others by having a long cytoplasmic tail of about 1000 amino acids instead of around 50 amino acids that interact with the intermediate filaments. Conserved regions of TM and cytoplasmic tail of both α and β integrin subunits have been reported to be essential in the regulation of integrin activation. The integrin can be locked in an inactive state by disulfide-bounds in the TM domain. Constitutive activation of integrin can be maintained by mutation in TM region or truncation of tail sequence (Banno & Ginsberg, 2008). Binding of talin to the cytoplasmic part of the β subunit induces a series of conformational changes that induce formation of an active extracellular domain with high affinity for ligands (Ulmer *et al*., 2003, Vinogradova *et al*., 2002).

Collagens are the most abundant proteins in the mammalian body. The collagen family contains at least 13 different proteins. Collagen fibers and fibrils give the structural support that is needed in the skeletal tissue, blood vessel and in the extracellular matrix in different tissues in the body. Type I collagen is the most abundant of the collagens and is expressed predominantly in tissues like skin, tendons and in the ECM of the skeleton where it is the major protein of the bone matrix. Amino acid residues of the I domain of an integrin determine its ligand-binding specificity to different collagens (Dickeson *et al*., 1998, Nykvist *et al*., 2000). The α2I domain D219 has been suggested to be important for collagen I binding (Smith *et al*., 2000), while in the α1 I domain that has higher affinity for collagen IV the corresponding amino acid is R218. In α10 and α11 this position has

R241 and T238 respectively. In addition, α1β1 and α2β1 attach to some non-collagen ligands involved in cell adhesion, such as laminin 1 and 2. Furthermore, α1β1 can bind to the cartilage protein matrilin-1 (Makihira *et al*., 1999) and α2β1 to the cartilage protein chondroadherin (Camper *et al*., 1997). All ligands for α1β1 and α2β1, except chondroadherin, support cell spreading. α10β1 attaches to type II collagen (Camper *et al*., 1998), while α11β1 is known to attach to fibrillar collagen I-III (Popova *et al*., 2007).

The other five integrins with I domain (αD, αE, αM, αL and αX) (Rose *et al*., 2007) are leukocyte integrins. The leukocyte integrins bind to counter receptors, including E-cadherin and intercellular and vascular adhesion molecules (ICAMs or VCAM correspondingly). Some of them also attach to plasma proteins such as plasminogen and fibrinogen or iC3b in the complement system (Table 1).

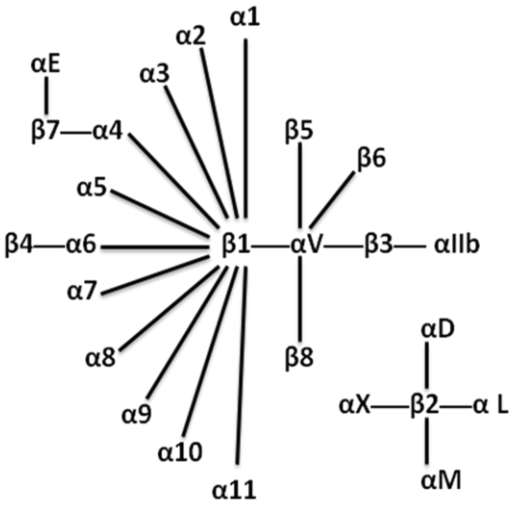


Figure 1. 18 α integrin subunits and eight β integrin subunits are able to form 24 heterodimeric integrins.

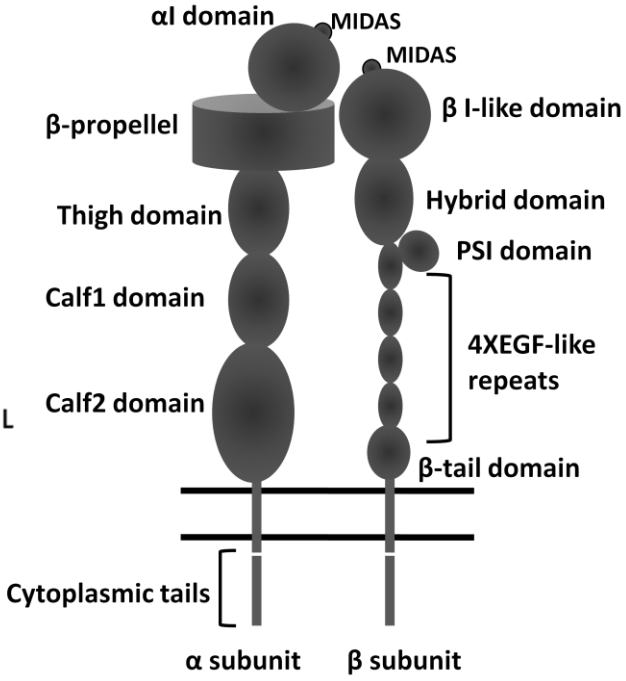


Figure 2. Schematic structure of collagen binding integrin. (Modified from Humphries, 2002; Hynes, 2002; White et al., 2004).

Several members of the integrin family, including α5β1, α8β1, αIIbβ3, αVβ3, αVβ5, αVβ6 and αVβ8, (Table 1), recognize an arginine-glycine-aspartic acid (RGD) tripeptide sequence in molecules such as fibrinogen, fibronectin, vitronectin, von Willebrand factor and laminins. Peptides containing the RGD motif, can efficiently block these integrin-ligand interactions (Hynes, 1992, Ruoslahti & Pierschbacher, 1987).

Other integrins containing neither the I domain nor the RGD sequence include the subunits α3, α4, α6, α7 and α9. The integrin subunits α3, α6 and α7 are all part of laminin-

binding integrins and are needed for the integrity of tissues such as kidney, skin and muscle (Table 1). α4 and α9 integrin subunits recognize ECM proteins, some plasma proteins, counter receptors belonging to the immunoglobulin superfamily and vascular endothelial cell growth factors (Vlahakis *et al*., 2007) (Table 1).

Outside-in signaling may be propagated by ligand binding to the extracellular domain, which initiates conformational changes that are mediated to the TM and cytoplasmic domains, and causes integrin clustering into discrete domains, such as focal adhesion domains (Arnaout *et al*., 2005). Integrin ECM site focal adhesion is formed by aggregation of several signalling and structural molecules, including focal adhesion kinase (FAK), Src, PI-3-Kinase, RhoGAP, paxillin, talin, p130CAS, integrin linked kinase (ILK) and Caveolin-1 in ECM (Gahmberg *et al*., 2009). The downstream signals following integrin ligation can modify cell function by altered cell morphology, cell migration or stability, regulation of cell differentiation and apoptosis. Inside-out signalling occurs by action of non-integrin cellular receptors or cytoplasmic molecules causing activation of signalling pathways inside the cell leading to either activation/deactivation of integrins.

Integrins have been implicated in various diseases and disease processes from inflammation, atherosclerosis to cancer. Altered expression of integrins in cancer cells contributes to metastasis tendency by influencing the cells' attachment to adjacent cells and their migration. Moreover, several viral pathogenes, including adenoviruses (Wickham *et al*., 1993), cytomegaloviruses (Feire *et al*., 2004), flaviviruses (La Linn *et al*., 2005), picornaviruses (Bergelson *et al*., 1992, Berinstein *et al*., 1995, Roivainen *et al*., 1994), rotaviruses (Ciarlet *et al*., 2002, Guerrero *et al*., 2000) and togaviruses (La Linn *et al*., 2005) use integrins as receptors to enter the host cell.

2.2 THE ROLE OF INTEGRINS IN BREAST CANCER METASTASIS TO THE SKELETON

Breast cancer is characterized by frequent skeletal metastasis. One possibility that might explain the common bone metastases is that the breast cancer cells express adhesion molecules that facilitate their adherence to the cell surface molecules present in the bone microvasculature matrix. Integrins mediate cellular adhesion and are crucial for the regulation of tissue integrity and several other functions in mammalian tissues. Integrins are involved in all steps of the metastasis process, including migration, invasion and colonization of target tissues (White & Muller, 2007), as well as in forming the vasculature crucial for tumor growth (Akalu *et al*., 2005, Goel & Languino, 2004).

The first stage of metastasis in a primary breast tumor is weakened adhesion to adjacent cells and migration from the primary tumor. This involves two properties that include forward movement of the cells and crossing the dense collagenous tissue

surrounding the tumor by degrading the extracellular matrix or, alternatively, squeezing through interstitial spaces (Wolf *et al*., 2003).

Integrins control the motility of cells. Migration movements include such as, synchronized processes of integrin engagement and actin cytoskeleton rearrangements (Giannone *et al*., 2004, Ridley, 2001). Integrin clustering at focal contact sites activates actin polymerization, mediated by members of the Rho family of small GTPases, including Rho, Rac and cdc42, providing the physical force for forward migration (Ridley *et al*., 2003). Furthermore, integrins are also implicated in the directionality of cell movement and breakdown of restrictive cell-cell adhesive structures. These properties are mediated through amplification of growth factor-derived signals (White & Muller, 2007).

Spreading of cells from the primary tumor requires simultaneous ability to degrade surrounding ECM barrier and to pass through the endothelial cell layer surrounding blood vessels. Degradation needs activation of membrane-bound or secreted proteases, generated by both tumor cells and inflammatory stromal cells. Matrix degradation includes membrane-bound or secreted proteases, such as members of the matrix metalloproteinase (MMP) family, which in invasive breast cancer cells are dependent on integrin expression (Baum *et al*., 2007, Brooks *et al*., 1996). Integrins have been shown to both promote MMP-mediated proteolysis and increase their expression (White & Muller, 2007). Inhibition of integrin expression in invasive breast tumor cells decreases MMP activity leading to reduction in invasive activity (Morini *et al*., 2000). Metastatic cancer cells have been reported to utilize integrins for attachment to the basement membrane in a distant environment (Van der Velde-Zimmermann *et al*., 1997, van der Pluijm *et al*., 1997). Basement membrane is composed of thin sheets of dense ECM enveloping all epithelial organs and functions as a barrier to macromolecules and cells (Albini *et al*., 2004).

Normal human breast epithelial cells express α1β1, α2β1, α3β1, α6β4 and αvβ3 integrins (Alford & Taylor-Papadimitriou, 1996, Damjanovich *et al*., 1997, Glukhova *et al*., 1995), whereas a common feature of breast tumors is altered expression of α1β1, α2β1, α3β1, α6β1 and α6β4 integrins (Alford & Taylor-Papadimitriou, 1996, Koukoulis *et al*., 1991).

αvβ3 integrin mediates adhesion of cancer cells and osteoclasts to the bone matrix (Harms *et al*., 2004). It can bind to an RGD peptide sequence present in many bone matrix proteins including vitronectin, osteopontin (OPN) and bone sialoprotein (Cacciari & Spalluto, 2005, Harms *et al*., 2004). Adhesive proteins, such as αvβ3, may mediate malignat cell addhesion to platelets (Nash *et al*., 2002), an association that could protect tumor cells from clearance by the immune system (Nieswandt *et al*., 1999). The αvβ3 integrin may also favour tumor cell adhesion to the vessel endothelium and their subsequent extravasation into distant tissues (Pontier & Muller, 2008).

The αvβ3 integrin has been shown to mediate certain cellular responses to OPN, a bone-derived phosphoglycoprotein related to aggressive disease progression and skeletal

metastasis (Furger *et al*., 2003). This can lead to upregulation of metastasis-promoting factors, such as MMPs and the Met/HGF receptor (Tuck *et al*., 2000). Induced expression of the αvβ3 integrin in malignant breast cancer has been shown to improve the survival of tumor cells after intravasation (Pontier & Muller, 2008). The αvβ3 integrin has also been implicated to be important in the metastasing process of breast cancer cells to bone. Breast metastatic cells and breast cancer-derived bone metastasis expressed high levels of the integrin (Liapis *et al*., 1996). In vivo -selected MDA-MB-231 cells that metastase only to bone show increased expression of the αvβ3 integrin (Hall *et al*., 2006). Inhibitors of the αvβ3 were shown to reduce bone metastasis in breast cancer patients and in an animal model after intracardiac injection of MDA-MB-435 cells (Harms *et al*., 2004). In a study with prostate cancer cells, blocking antibodies against the αvβ3 integrin inhibited attachment to crude bone protein extract by over 90% (Hullinger *et al*., 1998).

Furthermore, the αvβ3, together with the α5β1 integrin, has been implicated in breast cancer to promote proliferation signaling by interacting with growth receptor partners (Pontier & Muller, 2008). These events have been reported to regulate migration of malignant cells by coupling, for instance, to MMP9 metalloprotease (Felding-Habermann *et al*., 2001, Rolli *et al*., 2003). In animal models, administration of peptides designed to block the integrins α5β1 and αvβ3 impaired growth and metastasis of invasive breast cancer (Khalili *et al*., 2006, White & Muller, 2007).

Increase in the expression of the collagen/laminin receptor α2β1 has been reported in many-human malignancies (Jones *et al*., 1992, Pignatelli *et al*., 1990, Suzuki *et al*., 1993). In animal models, the α2β1 and the fibronectin receptor α4β1 have been shown to be responsible for selective metastasis of cancer cells to the skeleton (Chan *et al*., 1991, Matsuura *et al*., 1996). Controversially, reduction or loss of the α2β1 integrin has been observed in several breast cancers (Alford *et al*., 1998, Lanzafame *et al*., 1996) and it has associated with increased metastatic potential (Arihiro *et al*., 1993).

β1 integrin subunit expression is abnormal in approximately 30-50% of breast cancers and correlates with poor differentiation of the tumor (Cordes & Park, 2007). β1 integrin signaling has been observed to be involved in various stages of cancer progression, including invasion, migration and metastasis (Elliott *et al*., 1994, Fujita *et al*., 1995). The signaling depends on binding to extracellular ligands, including fibronectin and laminin-1. Decreased β1 integrin expression in breast tumors has been reported to correlate with more aggressive disease (Gonzalez *et al*., 1999, Lanzafame *et al*., 1996, Pignatelli *et al*., 1992). Conversely, increased β1 integrin signaling has also been reported to promote tumorigenesis by facilitating the activity of growth factor receptors (Wang *et al*., 1998, Wang *et al*., 2002). Inhibitory antibody against the β1 integrin induced apoptosis and decreased proliferation in three-dimensional cell cultures and in animal models of breast cancer (Yao *et al*., 2007). Overexpression of the β1 integrin was further reported to be associated with decreased survival in invasive cancer of breast (Yao *et al*., 2007) and

pancreas (Bottger *et al*., 1999), and cutaneous melanoma (Nikkola *et al*., 2004). The controversial tumor-promoting effect of both reduced and induced β1 integrin gene expression could be explained by different cellular regulation mechanisms (Yao *et al*., 2007).

Some other integrins have also been associated in the biology of breast cancer. β4 integrin-coupled signaling has been reported to be involved in breast cancer progression. α3β1 was suggested to be critical for migration, invasion and metastasis of a breast cancer cell line MDA-MB-231 to the skeleton (Morini *et al*., 2000) and the expression levels of the α*3*β1 integrin were higher in breast cancer metastases than in primary tumors. Furthermore, invasion was suggested to be dependent on the activity of MMP-9 (Morini *et al*., 2000). In an in vivo study, expression of signaling-defective β4 integrin in mammary epithelium delayed breast tumor progression in mice (Guo *et al*., 2006). The α6β1 and α6β4 integrins have been shown to contribute to breast cancer cell survival during cellular stress (Chung & Mercurio, 2004).

Some of the published results concerning the role of integrins in the breast cancer skeletal metastasis process are controversial, which could partly be explained by different surface expression of integrins on tumor cells. The αvβ3 integrin has been shown in numerous studies to be crucial for breast cancer cell attachment to bone surface. However, in the MDA-MB-231 cells that express low levels of the αvβ3 integrin, other integrins were suggested to be important in the metastasis to bone (Morini *et al*., 2000, Wong *et al*., 1998). Differences in experimental procedures may also affect the experimental results, for instance in studies by Morini et. al and Wong et al. showing the αvβ3 integrin not to be important in the initial attachment of MDA-MB-231 cells to bone (Morini *et al*., 2000, Wong *et al*., 1998). Pluijm et al. suggested the opposite in a study where cells were permitted to attach and spread on bone matrix over a long time span, possibly causing upregulation of additional surface integrins at the plasma membrane (van der Pluijm *et al*., 1997). In addition, factors other than the integrin expression profile could also affect the cell metastatic potential.

2.3 PICORNAVIRUSES

Picornaviruses are small (30 nm) nonenveloped animal viruses with a single-stranded infectious RNA genome. The family *Picornaviridae* is divided into twelve genera (aphto-, avihepato-, cardio-, entero-, erbo-, hepato-, kobu-, parecho-, seneca-, sapelo-, tescho- and tremoviruses) (http://www.picornaviridae.com) (Table 2). Every genus is further divided into species consisting of virus serotypes. Previously, picornavirus classification was based mainly on their pathogenesis in laboratory animals and antigenic properties (Hyypiä *et al*., 1997), but the current classification utilizes the genomic sequences of the

viruses. Picornaviruses are responsible for a diversity of diseases, such as poliomyelitis (polioviruses, PVs), common cold (human rhinoviruses; HRVs), hepatitis (hepatitis A virus), and foot-and-mouth-disease in cattle (foot-and-mouth-disease viruses; FMDVs) (Table 2).

Enteroviruses are among the most common viruses infecting humans globally. Human enteroviruses (HEVs) have former been classified into echoviruses (EVs), coxsackie A viruses (CAVs), coxsackie B viruses (CBVs) and polioviruses (PVs). In the current classification, HEVs are grouped into seven species: enterovirus A, B, C, and D, and rhinovirus A, B and C (Table 1). Enteroviruses are associated with a wide variety of illnesses, in all age groups, ranging from minor febrile disease to more severe conditions, including poliomyelitis, meningitis, encephalitis, myocarditis and neonatal generalized infection (Li *et al*., 2007). Most infections are, however, mild or asymptomatic (Grist *et al*., 1978). The principal site of infection is the epithelium of the respiratory or the gastrointestinal tracts. This can be followed by viremia (occurrence of viruses in the blood) that may cause transfer of the infection to secondary target organs. Enteroviruses have also been suggested to be involved in the development of some chronic diseases, such as type 1 diabetes (T1D) (Hyöty & Taylor, 2002) and dilated cardiomyopathy (Knowlton, 2008).

PVs were discovered as early as in 1908 and are the best-known enteroviruses (Landsteiner & Popper, 1909). PV infection can cause poliomyelitis which can lead to permanent paralysis. The virus has infected human populations for thousands of years. Although the poliovirus vaccination campaign has almost eradicated the disease, it is still a problem in some parts of the world. On the basis of coxsackievirus pathogenicity in laboratory animals, they have originally been divided into subgroups A and B (Dalldorf & Sickles, 1948). All CBVs can be propagated in cell culture, while some CAVs can only replicate in newborn mice. Echoviruses (enteric, cytopathogenic, orphan viruses) were originally recognized due to their ability to replicate exclusively in cell culture, in contrast to other enteroviruses which infected laboratory animals (Anonymous, 1955).

Oncolytic enteroviruses are potential agents for future cancer therapy. In 2004 Shafren et al. reported that CAV21 induced rapid oncolysis of human melanoma cells, overexpressing the virus receptors DAF and ICAM-1 in vitro and in vivo in mice bearing multiple melanoma xenografts (Shafren *et al*., 2004). This study was followed by additional reports implicating CAV21 to posses oncolytic potential in multiple melanoma (Au *et al*., 2007), breast tumors (Skelding *et al*., 2009) and prostate tumors (Berry *et al*., 2008) both in vitro and in vivo. CAV21 administrated to end-stage melanoma patients showed no adverse effect (Parato *et al*., 2005). In 2005 Shafren et al. showed EV1 to be oncolytic for cultured malignant ovarian cells expressing high levels of α2β1 integrin, but not for nonmalignant control cells (Shafren *et al*., 2005). The oncolytic effect was further determined by using human ovarian cancer xenografts in mice in which the tumor burden

was rapidly reduced, and in prostate and gastric cancer models (Berry *et al*., 2008, Haley *et al*., 2009, Shafren *et al*., 2005).

Table 2. Contemporary classification of picornaviruses (http://www.picornaviridae.com)

Genus	Species (No. of serotypes)
Enteroviruses	**Human enterovirus A** (21 types) **(HEV-A)**
	-Human coxsackieviruses A2-A8, A10, A12, A14, A16
	-Human enterovirus 71
	-Human echoviruses 76, 89, 90, 91
	-Simian enteroviruses enterovirus 92, SV19, SV43, SV46, A13
	Human enterovirus B (59 types) **(HEV-B)**
	-Human coxsackieviruses B1-B6
	-Human coxsackieviruses A9
	-Human echoviruses 1-7, 9, 11-21, 24-27, 29-33
	-Human enteroviruses 69, 73-75, 77-88, 93, 97-98, 100-101, 106-107
	-Simian enterovirus SA5
	Human enterovirus C (19 types) **(HEV-C)**
	-Human coxsackieviruses A1, A11, A13, A17, A19, A20-A22, A24
	-Human poliovirus 1-3
	- Human enteroviruses 95-96, 99, 102, 104-105, 109
	Human enterovirus D (3 types) **(HEV-D)**
	-Human enteroviruses 68, 70, 94
	Simian enterovirus A (3 types)
	Bovine enterovirus (2 types)
	Porcine enterovirus B (2 types) **(PEV-B)**
	Human rhinovirus A (74 types)
	-Human Rhinovirus 1-2, 7-13, 15-16, 18-25, 28-34, 36, 38-41, 43-47, 49-51, 53-68, 71, 73-78, 80-82, 85, 90, 94-96, 98, 100
	Human rhinovirus B (25 types)
	-Human Rhinovirus 3-6, 14, 17, 26-27, 35, 37, 42, 48, 52, 69, 70, 72, 79, 83, 84, 72, 79, 83-84, 86, 91-93, 97, 99
	Human rhinovirus C
Cardiovirus	**Encephalomyocarditis virus** (1 type)
	Theilovirus (12 types)
Aphtovirus	**Foot-and-mouth disease virus** (7 types)
	Equine rhinitis virus (1 type)
	Bovine rhinitis B virus
Hepatovirus	**Hepatitis A virus**
Parechovirus	**Human parechovirus** (14 types)
	Ljungan virus
Erbovirus	**Equine rhinitis B virus** (3 types)
Kobuvirus	**Aichi virus** (1 type)
	Bovine kobuvirus (1 type)
Teschovirus	**Porcine teschovirus** (11 types)
Sapelovirus	**Porcine sapelovirus**
	Simian sapelovirus
	Avian sapelovirus
Senecavirus	**Seneca Valley virus**
Tremovirus	**Avian encephalomyelitis virus**
Avihepatovirus	**Duck hepatitis A virus**

2.3.1 Structure of picornaviruses

The picornavirus genome is enclosed in an icosachedral capsid containing 60 copies of each of the four structural proteins VP1 to VP4. The capsid proteins bound together form the structural unit of the capsid, the protomer. Five protomers are arranged into a pentamer and 12 pentamers form the capsid. VP1, VP2 and VP3 remain external, while VP4 is buried within the capsid. The picornavirus genome is a positive-strand RNA molecule of 7.2 to 8.5 kilobases (kb) (Figure 3). It has a small virus-encoded protein (VPg) covalently linked to its 5' terminus, while the 3' end is polyadenylated. The 3' and 5' ends contain untranslated regions (5'UTR and 3'UTR, respectively). 5'UTR includes an internal ribosome entry site (IRES) and a cloverleaf secondary structure. IRES directs the initiation of translation in a cap-independent manner (Pelletier & Sonenberg, 1988), whereas the cloverleaf structure is a multifunctional replication element which interacts with viral and cellular proteins to form a ribonucleoprotein complex (Li *et al*., 2007). The 3'UTR contains a secondary structure, a pseudoknot, that has an important role in the regulation of viral RNA synthesis (Jacobson *et al*., 1993). The open reading frame (ORF) is translated into one single large polyprotein (between 2100-2400 amino acids, aa), which is further proteolytically cleaved into three precursor proteins P1, P2 and P3 and finally into four structural proteins (VP1-4) and seven nonstructural proteins (2A-C, 3A-D). 2A functions as a protease only in enteroviruses and rhinoviruses, and 3C in all picornaviruses (Hanecak *et al*., 1982, Toyoda *et al*., 1986). 3D is an RNA-dependent RNA polymerase.

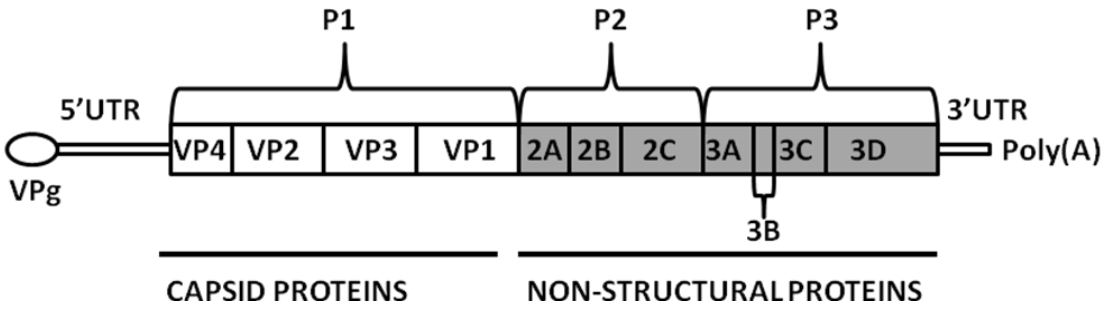


Figure 3. The enterovirus genome. The polyprotein is initially proteolytically cleaved into P1, P2 and P3 regions. The P1 region is processed into the structural proteins VP1-VP4, while P2 and P3 are processed into non-structural proteins.

2.3.2 Picornavirus infection cycle

To initiate infection, the virus needs to attach to a receptor on the cell surface (see step 1 in Fig. 4). The infection of most picornavirus infections is associated with major structural

modifications of the virion to the altered ('A') particle in order to release the viral RNA (vRNA). VP4 is absent in A particles, and the amino terminus of VP1 is externalized. For many enteroviruses, this structural change is known to be triggered by receptor binding (Rossmann *et al*., 2002). For other picornaviruses, such as the minor receptor group of HRVs, it is driven by the acidic pH within the endosomes (Dreschers *et al*., 2007). Enteroviruses are subsequently endocytosed and the viral genome is released into the cell cytosol (Pietiäinen *et al*., 2005) (see step 2 in Fig. 4). The VPg protein is removed from the 5'UTR in the cytoplasm and the genome is translated (see step 3 in Fig. 4) to a polyprotein precursor, which is then cleaved to provide the viral proteins crucial for genome replication and particle assembly particles (see step 4 in Fig. 4).

In picornaviruses, like in other RNA viruses, the virus genome synthesis occurs in replication complexes on membraneous vesicles that are induced by virus proteins (Egger *et al*., 2002). All the nonstructural proteins are involved in the viral RNA replication. In the initial step in replication, the positive strand viral RNA is copied to minus strand RNA (see step 5 in Fig. 4). This is followed by the production of new positive strand RNA molecules (see step 6 in Fig. 4). Enteroviruses inhibit cellular protein synthesis by cleaving the cellular initiation factor eIF4G, thus preventing the binding of the 5' cap-binding initiator factor eIF4E to cellular mRNAs with specific activity of the 2A protein (Etchison *et al*., 1982, Gradi *et al*., 1998). Already 2 h after the initiation of the PV infection translation of nearly all cellular genes is replaced by viral mRNA translation (Etchison *et al*., 1982). However, the translation of the picorna viral genome is not affected because of the IRES that allows cap-independent translation of RNA (Dorner *et al*., 1984, Pelletier & Sonenberg, 1989). Alternatively, picornaviruses can dephosphorylate two low-molecular-weight cellular proteins 4E-BP1 and 4E-BP2, which are then able to bind to EIF4E and block its binding to EIF4G (Gingras *et al*., 1998). Furthermore, a picornavirus infection can inhibit cellular RNA synthesis by inhibiting the DNA-dependent RNA polymerases I, II and III (Clark & Dasgupta, 1990, Falk *et al*., 1990, Yalamanchili *et al*., 1996).

After sufficient synthesis of picornavirus capsid proteins and genomic RNA, the encapsidation of viral particles is initiated (see step 7 in Fig. 4). The viral particles are released by cell lysis (see step 8 in Fig. 4). The replication cycle of enteroviruses ranges from 5 to 10 h in cell culture. Approximately 50 000 new virus particles are produced in one cell but only 0.1-2% of them are infectious (Racaniello, 2001).

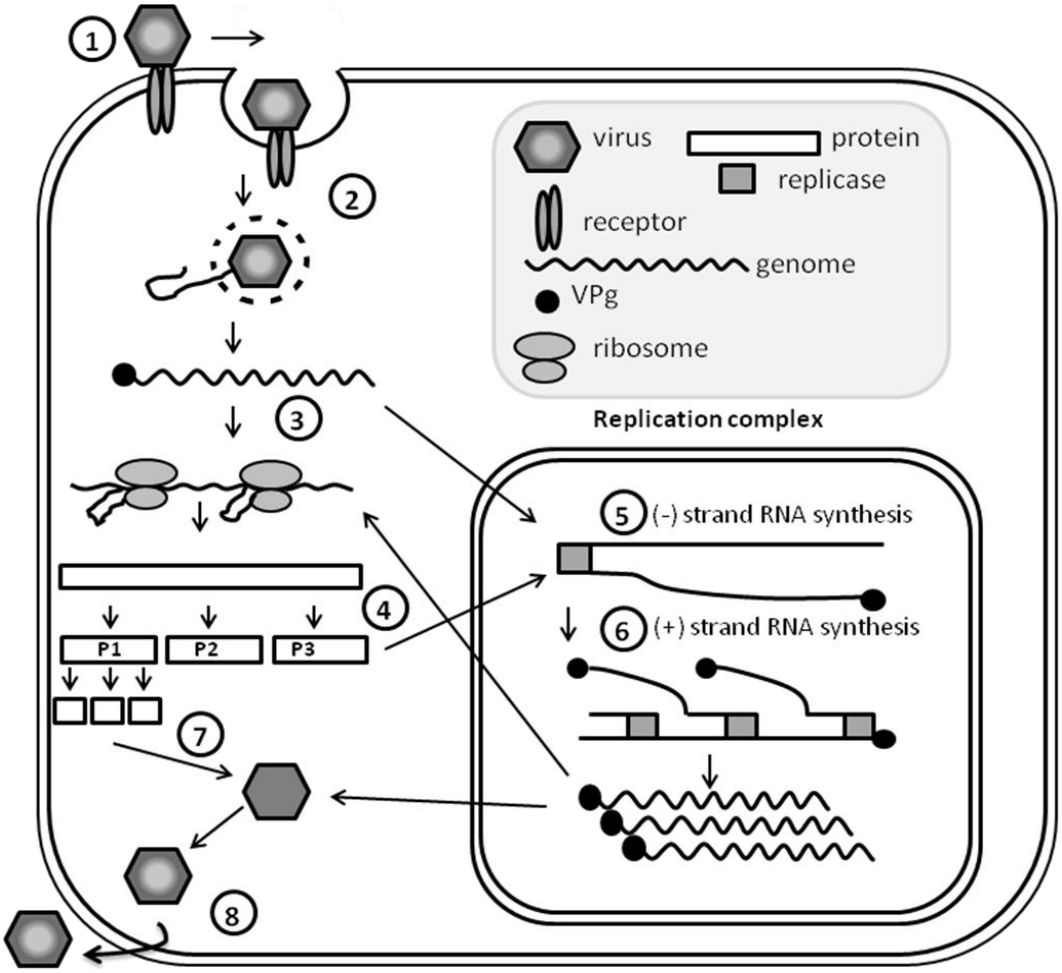


Figure 4. Schematic overview of the picornavirus replication cycle: 1) Attachment, 2) Entry and uncoating, 3) Translation, 4) Protein processing, 5-6) RNA replication, 5) (-) strand RNA synthesis, 6) (+) strand RNA synthesis, 7) Assembly and 8) Release.

2.4 CELLULAR RECEPTORS FOR PICORNAVIRUSES

To infect cells, viruses need to attach to molecules on the cell surface. Viruses use as receptors a wide range of molecules including proteins, carbohydrates and glycolipids. Virus-induced change in the receptor structure may induce signaling that activates the host-cell and regulates the entry process. The receptor binding can alter the virus structure triggering uncoating. Furthermore, accessory molecules may be needed for successful cell entry and virus uncoating. Picornaviruses may attach to several different molecules, including members of the immunoglobulin superfamily (IgSF) and integrins (Table 3 and Fig. 5).

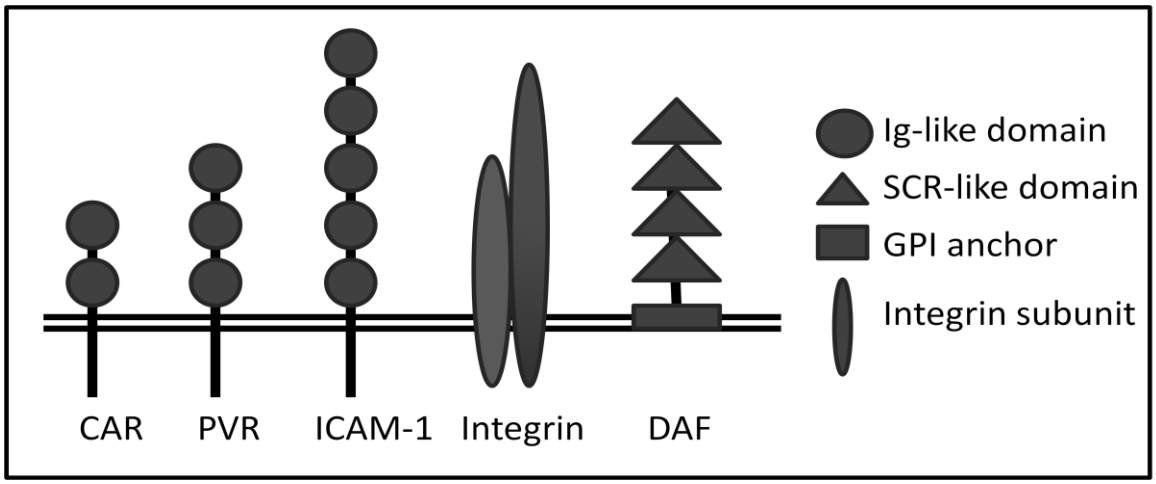


Figure 5. Schematic picture of proteins known to function as enterovirus receptors. (Modified from Evans and Almond, 1998).

Integrins are used as receptors by some picornaviruses. αv-integrin-binding picornaviruses, such as human parechoviruses (HPV1) (Joki-Korpela *et al*., 2001), CAV9 (Roivainen *et al*., 1994,Williams *et al*., 2004), some EV9 strains (Zimmermann *et al*., 1995) and FMDVs (Fox *et al*., 1989) posses an RGD motif in their VP1 capsid protein that functions as a binding site for αv-integrins (Chang *et al*., 1992, Fox *et al*., 1989) (Table 3). However, RGD-independent infection of CAV9 (Hughes *et al*., 1995, Roivainen *et al*., 1991, Roivainen *et al*., 1996) and FMDV (Jackson *et al*., 1996, Sa-Carvalho *et al*., 1997) has been reported, indicating that these viruses can use alternative receptors. Indeed, FMDV with mutated RGD sequence uses as receptor heparan sulphate (HS) (Baranowski *et al*., 2000).

Glucose-regulated protein 78 (GRP78, a member of the heat shock protein-70 family), is a coreceptor for CAV9 (Triantafilou *et al*., 2002) while MHC class I (Triantafilou *et al*., 2002) and beta2 microglobulin (Triantafilou *et al*., 1999, Heikkilä *et al*., 2010) participates in the endocytotic process of the virus. In HPEV1 infection, the RGD motif has been shown to be critical for the infection initiation (Boonyakiat *et al*., 2001), other molecules like MMP9 may participate in the entry process (Pulli *et al*., 1997). In addition, several non-RGD EVs were recently shown to attach to αv integrins (Ylipaasto *et al*., 2010) (Table 3).

The α2β1 integrin (collagen and laminin receptor) functions as a receptor for EV1 (Bergelson *et al*., 1992, Bergelson *et al*., 1993, Ohman *et al*., 2001). The α2I domain can exist in either closed or open conformation (Emsley *et al*., 2000). The open conformation occurs in activated integrins prior to ligand binding, suggesting that it represents a high avidity state of the α2I domain. EV1 and collagen interactions with the α2I domain differ considerably. The MIDAS amino acids and concomitant supporting by $Mg2^{+}$ are crucial for collagen attachment to the α2I domain, while these factors are not necessary in EV1

attachment to the integrin (Bergelson *et al*., 1993, King *et al*., 1997). Collagen, like all natural ligands, favours binding to the open α2I domain (Aquilina *et al*., 2002, Tulla *et al*., 2001) and α2I domain binding triggers conformation change from closed to open conformation (Emsley *et al*., 2000). Furthermore, clustering, either by collagen or antibody to the α2β1, triggered rapid and transient activation of p38 MAPK (Jokinen *et*

Table 3. Examples of picornavirus host cell receptors (Joki-Korpela et al., 2001, Racaniello, 2001, Williams et al., 2004, Ruiz-Saenz *et al*., 2009, Triantafilou *et al*., 2002,Yamayoshi *et al*., 2009, Yang *et al*., 2009,Ylipaasto *et al*., 2010).

RECEPTOR(S)	**Virus**
Integrins	
α2β1 integrin	Echovirus 1
αvβ3 integrin	Echovirus 25, 30, 32; Coxsackievirus A9
αvβ6 integrin	Coxsackievirus A9
αvβ1 and αvβ3 integrin	Human parechovirus 1
αvβ1, αvβ3, αvβ6, αvβ8 and α5β1 integrin	Foot-and-mouth disease virus
IgSF-like	
Poliovirus receptor, PVR; CD155,	Polioviruses
Intercellular adhesion molecule 1, ICAM-1; CD54	Coxsackieviruses A13, A18, A21
	Major receptor group of rhinoviruses
Coxsackievirus-adenovirus receptor, CAR	Coxsackieviruses B1-B6
HAV cellular receptor 1, HAVcr-1	Hepatitis A virus
SRC-like	
Decay-accelerating factor, DAF; CD55	Coxsackieviruses A21; Echoviruses 3, 6, 7, 11-13, 20, 21, 24, 29, 30
	Enterovirus 70
	Coxsackieviruses B1, B3, B5
Others	
Sialic acid	Enterovirus 70, 71
	Rhinovirus 87
Heparan sulphate	Foot-and-mouth disease virus (culture adapted)
	Certain echovirus serotypes
Low-density lipoprotein receptor; LDL-R	Minor receptor group of rhinoviruses
β2 microglobulin, β2m	Certain echovirus serotypes
	Coxsackievirus A9
Glucose-regulated protein 78	Coxsackievirus A9
Scavenger receptor class B, memBer 2, SCARB2	Enterovirus 71
SA-linked glycans	Enterovirus 71

al., 2010). EV1, unlike any other ligand known, preferred binding to the closed α2I domain and inactive α2β1 integrin (Jokinen *et al*., 2010, Xing *et al*., 2004). EV1-induced α2β1 integrin clustering did not significantly activate the p38 MAPK signaling cascade (Jokinen *et al*., 2010). Instead, EV1 clustering activated PKCα, which previously was shown to be needed for EV1 endocytosis (Upla *et al*., 2004). This suggested that EV1 induced clustering does not trigger similar conformational change of α2β1 integrin, caused by either collagen or antibodies (Jokinen *et al*., 2010).

In Cryo-EM reconstruction of EV1 interactions with a α2I domain-GST fusion protein, the α2I domain was shown to attach to VP1, VP2 and VP3 in the canyon of the virus. The MIDAS motif faced the canyon floor, although not in close contact with the virus, making it impossible for the α2I domain to bind collagen and virus simultaneously. Virus binding to α2I domain did not, however, induce virus uncoating. EV1 was able to attach simultaneously to multiple GST-α2I fusion proteins supporting the observation that EV1 attachment induces α2β1 clustering (Xing *et al*., 2004).

Immunoglobulin superfamily. Poliovirus receptor (PVR; CD155), coxsackievirus-adenovirus receptor (CAR), ICAM-1 (CD54), and hepatitis A virus cellular receptor 1 (HAVcr-1) are members of the IgSF and function as receptors for many picornaviruses, including PVs, CBVs, major group HRVs, CAVs and hepatitis A virus (Table 3). These proteins have an extracellular part, consisting of a variable number of Ig-like domains with disulphide bonds, a transmembrane domain and a short cytoplasmic domain. The N-terminal part of these receptors binds to the canyon in the virus particle (Rossmann *et al*., 2002).

All the three poliovirus serotypes utilize the PVR (Mendelsohn *et al*., 1989). There are two different functional PVRs, produced by alternative splicing, that differ only in the cytoplasmic sequence. PVR contains three extracellular IgSF domains (D1, D2 and D3) (Chothia & Jones, 1997) (Fig. 5). In folding D1 exhibits similarities with Ig variable domain, whereas the two other domains resemble immunoglobulin constant domains. Residues in the domain D1 of PVR have been reported to be involved in PV binding (Rossmann *et al*., 2002). The cytoplasmic domain of PVR attaches to the light chain of dynein motor complex and is suggested to transport vesicles containing intact PV in retrograde axonal transport to the neuronal-cell body (Mueller *et al*., 2002, Ohka *et al*., 2004).

ICAM-1 is recognized by members of the HRV major receptor group (Table 3), e.g. HRV14 and 16 (Greve *et al*., 1989, Staunton *et al*., 1989), and is also used by some CAVs, e.g. CAV 21 (Newcombe *et al*., 2003, Shafren *et al*., 1997a). ICAM-1 forms cell adhesions between leukocytes and endothelium by binding to lymphocyte function-associated antigen-1 (LFA-1; CD11a/CD18), a member of the integrin family, and the plasma protein fibrinogen, (Marlin & Springer, 1987). ICAM-1 contains five IgSF domains (Fig. 3). HRVs and CAV21 attach to the first aminoterminal IgSF of ICAM-1

(Kolatkar *et al*., 1999, Olson *et al*., 1993). Although the binding site of ICAM-1 to HRVs and CAV 21 canyons differs (Rossmann *et al*., 2002, Xiao *et al*., 2005), the interaction in both viruses induces conformational changes that destabilize the viruses and enhance the releases their genomes (Rossmann., 1994).

CAR functions as a cellular receptor for all CBVs (Bergelson *et al*., 1997, Tomko *et al*., 1997). It has two IgSF domains (Fig. 5) and is localized in intercellular contact sites in epithelial cell junctions, thus interacting in the regulation of the flow of ions and macromolecules, including viruses, across cell monolayers (Cohen *et al*., 2001). These tight junctions contain integral membrane proteins, which adher adjacent cell membranes in close contact with cytoplasmic scaffolding proteins that provide a contact to intracellular signaling molecules and cytoskeleton. An interesting aspect is that CAR is downregulated in some tumors (Fuxe *et al*., 2003, Okegawa *et al*., 2001, Sachs *et al*., 2002) and expression of transfected CAR causes inhibition of cell proliferation, suggesting that it could operate as a tumor suppressor (Anders *et al*., 2003). CBVes attach to several amino acids in the first domain of CAR (He *et al*., 2001) and virus binding to receptor induces conformational changes in the capsid (Goodfellow *et al*., 2005, Milstone *et al*., 2005).

Other picornavirus receptors. In addition to integrins and IgSF receptors, picornaviruses can use some others receptors, such as decay-accelerating factor (DAF), very-low-density lipoprotein receptor (VLDL-R), heparan sulphate (HS), sialic acids and β2 microglobulin (β2-m). Decay-accelerating factor (DAF) is a glycoprotein that participates in the regulation of complement activity (Nicholson-Weller *et al*., 1982) and can act as the cellular receptor for several echovirus serotypes (Bergelson *et al*., 1994, Clarkson *et al*., 1995, Powell *et al*., 1998, Ward *et al*., 1994), enterovirus 70 and as a secondary cellular receptor for CAV21 and CBV 1, 3 and 5 (Bergelson *et al*., 1995, Shafren *et al*., 1995) (Table 3). DAF is widely expressed in different mammalian cell types and contains four short consensus repeats (SRC) linked by a glycosylphosphatidylinositol (GPI) anchor (Medof *et al*., 1987) (Fig. 5). EV7 (Clarkson *et al*., 1995, He *et al*., 2002), EV12 (Bhella *et al*., 2004, Pettigrew *et al*., 2006), EV11 (Lea *et al*., 1998) and CBV3 (Bergelson *et al*., 1995) bind to regions near and of the third SRC domain, while enterovirus 70 (Karnauchow *et al*., 1996) and CAV21 (Shafren *et al*., 1997b) interacts with the first SRC domain. Virus-DAF interaction appears not to be sufficient to induce virus uncoating and A particle formation (Milstone *et al*., 2005).

The cellular receptors for the HRV minor receptor groups are members of the low density lipoprotein receptor (LDLR) family, including the low density lipoprotein receptor (LDLR), the very low density lipoprotein receptor (VLDLR) and LDLR related protein 1 (LRP1) (Nykjaer & Willnow, 2002, Vlasak *et al*., 2005). HS is a glycosaminoglycan ubiquitously expressed in mammalian cells. HS is naturally involved in cell adhesion, migration, proliferation, and differentiation. In addition, it binds to several signaling

molecules and a number of other ligands (Tumova *et al*., 2000). Some picornaviruses attach to HS, like FMDV (Jackson *et al*., 1996), swine vesicular disease virus (Escribano-Romero *et al*., 2004), CBV3 (Zautner *et al*., 2003), Theiler's murine encephalomyelitis virus (Reddi & Lipton, 2002), some EVs (Goodfellow *et al*., 2001) and HRV54 (Khan *et al*., 2007) (Table 3).

Sialic acids are a family of negatively charged sugar molecules located at the cell surface, most often on glycans of glycoproteins, glycosphingolipids, and the proteoglycan keratan sulphate (Angata & Varki, 2002). A couple of enteroviruses, including enteroviruses 70 (Alexander & Dimock, 2002, Haddad *et al*., 2004, Nokhbeh *et al*., 2005) and 71 (Yang *et al*., 2009) and RHV87 (Uncapher *et al*., 1991), have been reported to use sialic acids. β2-m associates with MHC class I molecules on the cell surface (Bjorkman *et al*., 1987, Madden, 1995)**.**

β2-m has been suggested in antibody inhibition studies to function as a receptor for CAV9 (Heikkilä *et al*., 2010, Triantafilou *et al*., 1999) and some EVs serotypes including EV1 (Ward *et al*., 1998).

2.5 INTERNALIZATION OF PICORNAVIRUSES INTO HOST CELLS

Table 4. Internalization pathways of enteroviruses.

Endocytic pathway	Virus	Reference
Chlathrin-dependent	**CBV3**	(Chung *et al*., 2005)
	EV6	(Leveque *et al*., 2007)
	PV	(Kronenberger *et al*., 1998, Willingmann *et al*., 1989)
	HRV2	(Brabec *et al*., 2003, Schober *et al*., 1998)
	HRV14	(Grunert *et al*., 1997, Schober *et al*., 1998)
	HRV16	(Lau *et al*., 2008)
	HPEV1	(Joki-Korpela *et al*., 2001)
Lipid raft /Caveolae-dependent	**CAV9**	(Triantafilou & Triantafilou, 2003)
	CBV4	(Triantafilou & Triantafilou, 2004)
	EV1	(Karjalainen *et al*., 2008, Marjomäki *et al*., 2002, Pietiäinen *et al*., 2004)
	EV6	(Leveque *et al*., 2007)
	PV	(Coyne *et al*., 2007a)
Macropinocytosis	**CAV9**	(Heikkilä *et al*., 2010)
	CBV3	(Coyne *et al*., 2007b)
	EV1	(Karjalainen *et al*., 2008, Marjomäki *et al*., 2002, Pietiäinen *et al*., 2004)
Other	**PV**	(Brandenburg *et al*., 2007)
	HRV14	(Khan *et al*., 2010)

To infect the cell picornaviruses must penetrate the plasma membrane. Most nonenveloped viruses, like picornaviruses, use endocytosis for host cell entry. Picornaviruses enter the host cell by clathrin-dependent and lipid raft/caveolin-dependent pathways and by macropinocytosis (Table 4.). Many viruses are able to switch from one uptake mechanism to another or simultaneously utilize more than one endocytic route (Sieczkarski & Whittaker, 2005).

2.5.1 Clahrin-dependent endocytosis

Clathrin-mediated endocytosis is essential for several cellular mechanisms, including nutrient uptake, synaptic vesicle recycling, and transport of receptors and ion channels from the cell membrane (Brodsky *et al*., 2001, Cremona & De Camilli, 1997, Hirst & Robinson, 1998, Marsh & McMahon., 1999). The clathrin molecule has a conformation of a triskelion with three kinked extensions that radiate from a central vertex (Kirchhausen, 2000). Clathrin-coated pits concentrate cargo proteins for internalization (Kirchhausen, 2000), and internalization can occur constitutively or in response to stimuli. Several proteins, including adaptor complex AP-2, dynamin GTPase, neuronal adaptor protein AP180, CALM (Nonet *et al*., 1999, Zhang *et al*., 1998) and Eps 15 regulate the assembly and fission of the pits (Benmerah *et al*., 1999, Chen *et al*., 1999). Dynamin GTPase catalyzes vesicle fission upon hydrolysis of GTP (Mettlen *et al*., 2009). The internalized clathrin-coated vesicle is rapidly uncoated and fuses with early endosomes (EEs) (Chappell *et al*., 1986). Some ligands detach from their receptors in the slightly acidic milieu of EEs. Endocytosed receptors can be recycled back to the plasma membrane from EEs in a fast (approximately 5 min) or a slow (approximately 15-30min) recycling pathway. In the latter the cargo is first shuttled to endocytic recycling compartment (ERC) before transport to recycling endosome (RE), while in fast recycling the cargo enters directly from EE in RE. Alternatively, the cargo can be transported from EEs to late endosome (LE), with a lower pH in 5 to 15 minutes. The transport from EE to LE has been suggested to be mediated by an endosomal carrier vesicle (ECV) (Clague *et al*., 1994, Gu & Gruenberg, 1999, Gu & Gruenberg., 2000). Some cargoes are transported from LEs into lysosomes, which have an acidic pH and hydrolytic enzymes for degradation (Fig 6.).

Rab proteins belong to the Ras superfamily of GTPases, which includes four other families; Ras, Rho, Arf and Ran (Garcia-Ranea & Valencia, 1998, Takai *et al*., 2001). Ras and Rho have a role in cell growth and motility, while Rab, together with the latter two, regulates intracellular trafficking. Several Rabs are involved in clathrin-dependent endocytosis in endosomal vesicles (Table 5). Examples of other important molecules in the chlathrin-dependent entry route include the Rab5 effectors, EEA1 and PI(3)K, needed

in EE fusion. EEA1 functions as a tethering protein between two Rab5 positive membranes (Rothman & Warren, 1994).

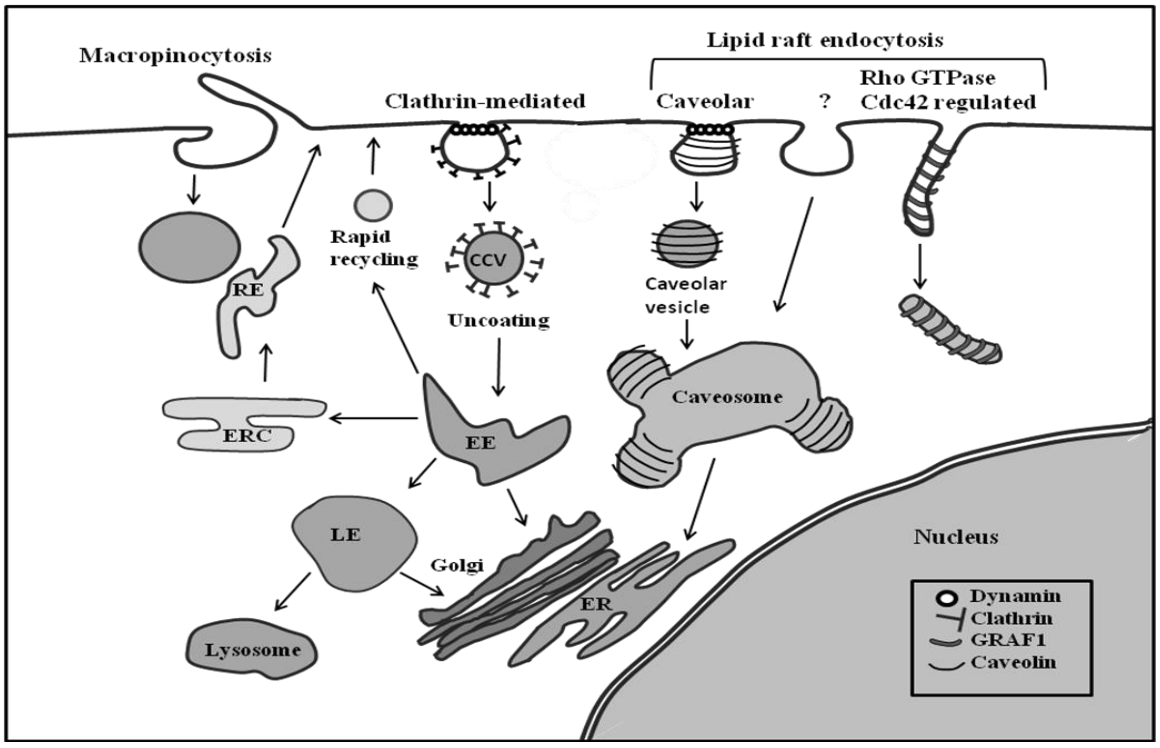


Figure 6. Endocytic pathways. Internalization pathways for cargo include macropinocytosis, clathrin mediated endocytosis and lipid raft endocytosis mechanisms.

Transferrin (Tf) transport is a widely used marker for clathrin-mediated entry. Tf belongs to a family of iron-binding proteins. The iron-Tf complex binds to the transferrin receptor (TfR) and is rapidly endocytosed through clathrin-coated pits (Mellman, 1996). TfR is constitutively endocytosed, and the rate of recycling is further enhanced by Tf binding. From the clathrin-coated vesicle the Tf-TfR is transported to EEs. In the endosomal acidic environment of the EE, iron is released from Tf and the resultant apo-Tf-TfR is recycled to the plasma membrane (Hedman *et al*., 1987, Morgan, 1996, Qian *et al*., 1997) in either a rapid or slow recycling route (Grant & Donaldson, 2009).

Several viruses, including adenoviruses, alphaviruses, hantaviruses, orthomyxoviruses, parvoviruses and picornaviruses (DeTulleo & Kirchhausen, 1998, Evans & Almond, 1998, Marsh & Pelchen-Matthews, 2000), utilize clathrin–mediated endocytosis. The incoming viruses are most often exposed to the acidic milieu of endosomes. Many viruses respond to the pH decrease by undergoing changes that lead to endosomal membrane penetration and viral genome release. Some enteroviruses (Table 4), aphthovirus, FMDV and parechovirus HPEV1 are picornaviruses that have been shown to use clathrin-mediated entry.

Table 5. Rab proteins involved in clathrin-dependent endocytosis.

Rab	Function	Reference
Rab 4	Rapid recycling from EE to plasma membrane	(Deneka *et al*., 2003, Yudowski *et al*., 2009)
Rab5	Transport from EE to LE	(Bucci *et al*., 1992, Gorvel *et al*., 1991)
Rab7	Transport from EE to LE for certain molecules	(Fan *et al*., 2003, Feng *et al*., 1995, Press *et al*., 1998)
	Transport from late LE to lysosome	(Bottger *et al*., 1996, Bucci *et al*., 2000, Meresse *et al*., 1995, Schimmoller & Riezman, 1993)
Rab10	Implicated to be involved in transport between EE and RE	(Babbey *et al*., 2006, Chen *et al*., 2006a)
Rab11	Transport from EE to ERC	(Naslavsky *et al*., 2006)
	Recycling from EE to plasma membrane	(Sheff *et al*., 1999)
	Transport from ERC to RE and recycling to the plasma membrane	(Weigert *et al*., 2004)
Rab22A	Transport from EE to ERC	(Magadan *et al*., 2006)
Rab35	Rapid recycling from EE to plasma membrane	(Kouranti *et al*., 2006)

2.5.2 Lipid raft/caveolae-mediated endocytosis

Lipid rafts are small (10-200 nm), heterogeneous, highly dynamic, sterol and sphingolipid-enriched domains that compartmentalize cellular processes (Pike, 2006). The main components of lipid rafts are sphingolipids, (glycol-) phospholipids, and cholesterol. Cholesterol partition into sphingolipid bilayer makes it more flexible and enables substantial lateral movement of the lipid rafts in the membrane (Simons & Ikonen, 1997). Small lipid rafts can form larger platforms by protein-protein and protein-lipid interactions (Pike, 2006). Raft formation and/or stabilization is thought to be a highly regulated process that occurs only in response to activation by a stimulus, e.g. receptor clustering (Hammond *et al*., 2005). Specific proteins, such as caveolins (Mukherjee *et al*., 1999) and flotillins (Rajendran *et al*., 2003) are thought to organize rafts into specialized membrane domains.

Lipid rafts have a role in the antigen presentation (Gombos *et al*., 2004, Gombos *et al*., 2006), signal transduction and act in sorting of trans-Golgi network (Keller & Simons, 1998, Simons & van Meer, 1988) and endosomes (Mukherjee *et al*., 1999). Many pathogens, including bacteria (Lafont & van der Goot, 2005) and viruses (Chazal & Gerlier, 2003, Pelkmans, 2005), have evolved to use lipid rafts for their own internalization.

Several receptors and signaling proteins (e.g. Src, Lck, and CD4) are associated with lipid rafts, while others colocalize upon activation (Gupta & DeFranco, 2003, Kabouridis

et al., 1997). Some proteins that lack transmembrane spans are targeted to lipid raft domains, particularly proteins with GPI-anchors (Brown & London, 1998, Gombos *et al*., 2008). Transmembrane proteins are generally excluded from tightly packed lipid raft structures (Fastenberg *et al*., 2003, van Duyl *et al*., 2002). Nevertheless, transmembrane proteins can be directed to lipid rafts by addition of raft targeting signals. At least four different raft-dependent pathways are thought to exist in mammalian cells. These pathways differ in their requirements for dynamin and small GTPases organizing the endocytosis and cargo selection. Two of the pathways are dynamin-dependent, and of those two, one is regulated by caveolin and the other is dependent on RhoA. The two other dynamin-independent lipid rafts are controlled by Rho GTPases Cdc42 and GTPase Arf 6 respectively (Upla *et al*., 2009).

Caveolae are flask-shaped invaginations in the plasma membrane that contain sphingolipids, cholesterol, polymerase I and transcript release factor (PTRF)-cavin and caveolin proteins (Anderson, 1998, Hill *et al*., 2008, Simons & Ikonen, 1997). The caveolin proteins are thought to form a hairpin structure in the membrane. They have a central 33-amino acid long hydrophobic domain, with both the N-terminus and C-terminus facing the cytoplasm (Dietzen *et al*., 1995, Dupree *et al*., 1993, Monier *et al*., 1995). Cholesterol binds tightly in a 1:1 ratio to caveolin, and it is crucial for stabilizing of the caveolin oligomers (Monier *et al*., 1996) and involved in signaling and trafficking mechanisms of caveolae.

Caveolae have been implicated in several functions including cell signaling, vesicular transport and lipid regulation. Caveolar endocytosis is used by natural ligands (e.g. albumin, cholesterol) (Schnitzer *et al*., 1994, Smart *et al*., 1996), toxins (e.g. cholera toxin; CTx) (Montesano *et al*., 1982, Nichols, 2002), bacteria (e.g. E. coli) (Shin *et al*., 2000), viruses (Pelkmans & Helenius, 2002) and prions (Peters *et al*., 2003). Due to the important regulatory role of caveolae in several signaling cascades, mutations or defects in caveolin proteins contribute to the pathogenesis of some diseases, including type 2 diabetes, cancer, cardiovascular diseases, atherosclerosis, pulmonary fibrosis and some types of degenerative muscular dystrophies (Schwencke *et al*., 2006).

Caveolae have been suggested to be involved in the regulation of various pathways, including those regulated by integrins, by clearing proteins from the plasma membrane (Pelkmans *et al*., 2005). Integrins regulate multiple pathways, some of which can be constitutively activated in cancer cells by the lack of cellular regulation by caveolin-1. Caveolin-1 expression is suppressed in some oncogenically transformed cells, leading to the absence of detectable caveolae (Koleske *et al*., 1995). For instance, decreased caveolin-1 expression in breast cancer cells contributes to higher invasive potential (Sloan *et al*., 2004). Reexpression of caveolin-1 in tumor cells restores anchorage-dependent growth (Engelman *et al*., 1997).

Caveolae are partially immobile in the plasma membrane, and activation by ligand binding to receptor may trigger internalization. Tyrosin phosporylation of caveolin-1 can activate caveolae endocytosis. Internalization activation temporarily recruits GTP-binding protein dynamin to caveolae, triggering fission of caveolae by subsequent hydrolysis of GTP and constriction of caveolae neck. Internalization of caveolae is further dependent on reorganization of the cytoskeleton by depolymerization of actin (Parton *et al*., 1994). After pinching off from the plasma membrane, caveolae may connect to microtubule-associated proteins for transport (Schnitzer *et al*., 1995). The cytoplasmic caveolae can fuse with caveosomes (Pelkmans *et al*., 2001) or alternatively deliver their cargo to endosomal vesicles of the clathrin-mediated pathway (Pelkmans *et al*., 2004). Caveosomes have a neutral pH, and they are caveolin-1 positive and rich in cholesterol and glycosphingolipids (Pelkmans *et al*., 2001). The cargo can be delivered from caveolar vesicles and caveosomes further to the endosomes, Golgi, ER or lysosoms, a process that may involve microtubule-directed transport (Conrad *et al*., 1995, Nichols, 2002).

Virus entry by caveolar endocytosis. Since SV40 was shown to enter the cell by caveolar endocytosis (Anderson *et al*., 1996), a growing number of viruses, including the enteroviruses EV1 (Marjomäki *et al*., 2002), PV (Coyne *et al*., 2007a), respiratory syncytial virus (Brown *et al*., 2002) and filoviruses (Empig & Goldsmith, 2002) were shown to utilize caveolin-mediated endocytosis. A benefit for viruses to use caveolae entry is that they avoid the acidic endosomes/lysosomes. The caveolae-dependent endocytosis transports the pathogens to a specific destination, e.g. SV40 to the endoplasmic reticulum (Pelkmans *et al*., 2001), cholera toxin to the Golgi compartment (Nichols, 2002) and EV1 to the perinuclear space (Marjomäki *et al*., 2002). EV1 attaches to the host cell by binding to the α2 I domain (α2I) of the α2β1 integrin on the cell surface (Bergelson *et al*., 1992). In former studies, the virus has been shown to internalize together with the integrin receptor in either caveolin-1 positive or negative vesicles and is later transported to caveosomes (Marjomäki *et al*., 2002, Pietiäinen *et al*., 2004). The virus infection was shown to be dependent on dynamin-2 in some cell lines (Karjalainen *et al*., 2008, Pietiäinen *et al*., 2004) and partly on cholesterol (Marjomäki *et al*., 2002, Pietiäinen *et al*., 2004), and the need of an intact actin cortex was shown to vary according to cell type (Pietiäinen *et al*., 2004). However, later studies indicate that the majority of the viruses use alternative entry process that is independent of both clathrin and caveolin.

Picornaviruses utilizing both lipid raft/caveolae and clathrin entry routes. A few picornavirus serotypes, including CBV3, FMDV and EV6, have been shown to use both lipid raft/caveolin and clathrin-dependent routes of endocytosis, depending on cell type or genetic variation in the virus genome (Chung *et al*., 2005, Coyne *et al*., 2007b, Leveque *et al*., 2007, Wang *et al*., 1993). CBV3 were found to use clathrin-dependent entry in Hela cells (Chung *et al*., 2005), while another CBV3 strain infecting HeLa CCL-2 cells required dynamin and lipid rafts (Patel *et al*., 2009). In polarized epithelial cells, virus

entry from tight junction (TJ) is dependent on caveolin, Ras, Rab5 and Rab34 GTPases involved in macropinocytoses and independent of dynamin (Coyne *et al*., 2007b). FMDV that is virulent for susceptible animals has been shown to attach to five different integrins (table 3) (Ruiz-Saenz *et al*., 2009). Tissue culture adaption of some FMDV serotypes causes loss of virulence in animals and integrins as cellular receptors. Instead, those serotypes utilize cell surface HS molecules as receptors. In two strains of genetically engineered FMDV, the first was able to bind to both HS and integrin and entered the cells by both clathrin and caveolin-mediated entry, while the second that only utilized HS as cellular receptor colocalized with caveolin (ODonnell *et al*., 2008). The cell entry of two strains of EV6 was studied, where one had the ability to hemaglutinate human erythrocytes, a feature related to the virus attachment to DAF (Powell *et al*., 1999), while the other virus strain had lost its hemaglutinating capacity due to inability to attach to DAF. DAF are concentrated in the plasma membrane within the lipid rafts domain. DAF-binding EV6 strain did enter through a lipid raft-dependent pathway, while the non-DAF-binding strain endocytosed through clathrin-directed pathway (Leveque *et al*., 2007).

Other endocytotic pathways through lipid rafts. In addition to caveolae entry, at least three other lipid raft endocytotic pathways have been proposed to exist. These include a dynamin-dependent pathway regulated by RhoA. RhoA GTPases are important in the regulation of cytoskeletal dynamics and are connected to several cellular processes, including vesicular transport. The two other lipid rafts-mediated entry pathways are dynamin-independent. One of them is controlled by Rho GTPases Cdc42, while the other is regulated by GTPase Arf 6. Activity of Rac1, Cdc42, N-WASP and a GTPase regulator, connected with a focal adhesion kinase 1 upstream of Cdc42 (Lundmark *et al*., 2008), triggers the forming of acidic endocytotic vesicles called clathrin-independent carriers (CLICs) (Chadda *et al*., 2007, Kalia *et al*., 2006, Kumari & Mayor, 2008, Sabharanjak *et al*., 2002). The CLICs obtain Rab5 and EEA1 and fuse with other CLICs forming GPI-anchored, protein-enriched early endosomal compartments (GEECs). The GEECs subsequently fuse with the sorting endosomes of the clathrin pathway in a process that needs both PI3K and Rab5 (Kalia *et al*., 2006).

Arf6-dependent lipid raft recycling internalization pathways have been reported to be utilized by GPI-anchors and some integral membrane proteins, such as β1-integrins. The internalized molecules enter phosphatidylinositol-(4,5)-bisphosphate (PIP_2)- and cholesterol-containing membranes before being transported to the sorting endosomes of the clathrin pathway. The cargo can be further transported to recycling endosomes by a Rab22-dependent process or alternatively shuttled to the lysosomal vesicles.

Flotilins are raft-associated proteins that are located in microdomains and able to form membrane curvature and endocytosis (Babuke & Tikkanen, 2007, Frick *et al*., 2007, Glebov *et al*., 2006). The regulation mechanism for this entry process is still unsolved. EV1 have been reported to use an alternative entry process that is independent of both

clathrin and caveolin and in some cell types of dynamin. The virus attached to a lipid raft is internalized in endosomes that maturate further into multivesicular structures which recruit caveolin-1 by fusing with caveolae or caveosomal structures, thus forming structures that may be considered as late caveosomes (Karjalainen *et al*., 2008).

2.5.3 Macropinocytosis

By macropinocytosis, cells are able to take up large quantities of extracellular fluids and macromolecules. Some microbial pathogens also utilize this pathway to enter the cells. Macropinocytosis is either a stimulated or constitutively active process depending on the cell type. Dendritic cells take up antigens and, after processing them into peptides, present them to T-cells. Macropinocytosis is driven by actin polymerization causing circular ruffles of the plasma membrane to form large endocytotic macropinosome vesicles. Mostly macropinocytosis is growth factor-induced, although various molecules can induce ruffling independent of growth factors (Mercer & Helenius, 2009). Macropinocytosis can be induced by external stimulation, e.g. by growth factors that trigger activation of receptor tyrosine kinase (RTKs), which leads to a signaling cascade that alters the dynamics of actin filaments and results in ruffling. Ras superfamily GTPases are important in this process, and RTK activation of Ras activates three parallel signaling pathways containing Rac1, Rab5, Arf6 and phosphotidyl-3-kinase (PI(3)K) (Bar-Sagi & Feramisco, 1986, Bar-Sagi *et al*., 1987), which together cooperate to modulate ruffle formation, macropinosome closure and membrane transport (Lanzetti *et al*., 2004).

Several kinases are crucial for macropinocytosis, and one of the most important is the p21-activated kinase 1 (Pak1), a serine/threonine kinase activated by Rac1 and Cdc42. Pak1 is needed during all stages of macropinocytosis. It regulates cytoskeleton dynamics and motility and activates numerous effectors needed for membrane ruffling and macropinosome closure (Dharmawardhane *et al*., 2000, Liberali *et al*., 2008, Parrini *et al*., 2005). Other kinases, important for membrane ruffling and macropinosome formation are protein kinase C (PKC) (Amyere *et al*., 2000, Miyata *et al*., 1989) and c-Src. Na^+/H^+ exchangers are also important markers for the macropinosome (West *et al*., 1989).

Macropinocytosis has been reported in several studies to be used as an entry route by viruses, including vaccinia virus, rubella virus, human immunodeficiency virus type I (HIV-1), herpes simplex virus 1 (HSV1) and adenoviruses (Ad) (Mercer & Helenius, 2008). Some viruses are thought to use macropinocytosis as a direct endocytotic route, while others may use it indirectly to assist in viral infection and simultaneously use other endocytotic mechanisms (Mercer & Helenius, 2008). Two enteroviruses, EV1 and CBV3, have so far been suggested to use macropinocytosis in their entry. EV1 is attached to its receptor, the α2β1 integrin, and internalized into vacuolar structures. The infection was

shown to be dependent on several molecules known to be involved in macropinocytosis, such as Pak1, PI(3)K, PLC, Rac1 and Na^+/H^+ exchangers. The infection required cholesterol and actin dynamics (Karjalainen *et al*., 2008) and was further shown to be dependent on C-terminal-binding protein-1/brefeldinA-ADP ribosylated substrate (CtBP1/BARS) (Liberali *et al*., 2008). In polarized epithelial cells, CBV3 enters cells from epithelial TJ within macropinosomes (Coyne *et al*., 2007b). Virus entry was dependent on caveolin, Ras, Rab5 and Rab34 GTPases, Na^+/H^+ exchangers and PKC, but independent of dynamin (Coyne & Bergelson, 2006).

2.6 HOST GENE EXPRESSION INDUCED BY ENTEROVIRUS INFECTION

Enterovirus infections are able to cause dramatic change in the host cell. Certain enteroviruses, such as PV1 (Johannes *et al*., 1999), EV1 (Huttunen *et al*., 1997, Huttunen *et al*., 1998, Pietiäinen *et al*., 2000) and EV7 (Huttunen *et al*., 1997) have been shown to affect the expression of eukaryotic immediate early genes (IEGs). Many enteroviruses have been shown to control cell death by either activating or inhibiting apoptosis. In addition, in PV infected cells concomitant activation of both apoptotic and antiapoptotic pathways has been shown to occur (Autret *et al*., 2008).

Immediate early genes (IEGs) are a class of genes that are rapidly and usually transiently activated in response to a wide variety of cellular stimuli (Soloaga *et al*., 2003). The induction of IEGs occurs in the absence of de novo protein synthesis (Platenik *et al*., 2000). IEGs function for instance as chemo-attractants, cytoplasmic enzymes, ligand-dependent transcription factors and inducible transcription factors (ITFs), including the Fos protein family (FOS, FOSB, Fra-1, Fra-2), Jun (JUN, JUNB, JUND) (Herdegen & Leah, 1998) and some members of activating transcription factors (ATF) (e.g ATFa, ATF-2 and ATF2, ATF3). These genes are components of the dimeric AP-1 transcription factor that regulates a wide range of cellular processes, such as cell proliferation, cell survival and differentiation (Shaulian & Karin, 2002).

Several IEGs have been determined to be increased during EV1 infection. The transcription of IEGs JUN, JUNB, and FOS has been shown to increase 5-10 h p.i. in EV1-infected cells in a Northern blot analysis (Huttunen *et al*., 1997) and in studies measuring the transcriptional rate of the genes (Huttunen *et al*., 1998). EV1 infection causes activation by phosphorylating both the stress-related p38 mitogen-activated protein kinase (MAPK) and the growth signal-related ERK1/2 MAPKs. The growth signal-related ERK1/2 MAPKs pathway leads to induction of FOS, whereas p38 MAPK were involved in the increased expression of JUNB (Huttunen *et al*., 1998). Furthermore, in a microarray study, EV1 infection induced expression of two IEGs, Fos related antigen (Fra-1) and

early growth response 1 (EGR1) 6 h p.i. 10 h p.i induction of both of these IEGs was even more strongly induced and the expression of a few additional IEGs was increased, including JUN, Krueppel-like factor 10 (KLF10) and c-myc (Pietiäinen *et al*., 2000). α2β1 integrin–mediated cell adhesion to type I collagen is known to induce certain IEGs. However, IEGs activation has not been observed during early time points of virus attachment and endocytosis, suggesting viral replication to be essential for the induction of these genes (Huttunen *et al*., 1997, Pietiäinen *et al*., 2000). This view was supported by the fact that transfection of cells with viral EV1 RNA induced the activation of JUNB and that the induction of FOS, JUN and JUNB were also observed in EV7 infected HOS pα2AW cells (Huttunen *et al*., 1997).

Similar pattern of induction of IEGS in host cells has been shown to occur during the infection of other enteroviruses. In PV-infected Hela cells, the expression of some IEGs was found to be increased upon the infection (Johannes *et al*., 1999). These IEGs included ATF3, c-myc, cysteine-rich, angiogenic inducer, 61 (CYR61), Pim-1 and KLF10. In another study, PV-infection also induced increased expression of Pim-1, c-myc and JUN (Pietiäinen *et al*., 2000). In line with these studies, CBV3 infection was found to transiently upregulate ATF3, JUN, FOS (McManus *et al*., 2002), CYR61 and KLF10 (Kim *et al*., 2004). Furthermore, the gene expression profile of CBV3-infected mouse hearts showed upregulation of the expression of ATF4, ATF3 and ATF4 (Shaulian & Karin, 2002)**.** They can act as transcriptional repressors or attach to JUN in order to induce cellular transcription. In a murine model of chronic CBV3 myocarditis, the expression of connective tissue growth factor (CTGF) was found to be highly induced (Lang *et al*., 2008).

Apoptosis-related genes. Apoptosis is an active process of cell death characterized by DNA fragmentation, nuclear chromatin condensation, membrane blebbing and cell shrinkage (Arends *et al*., 1990). Caspases are molecules involved in apoptosis, which belong to a family of cysteine proteases located mainly in the cytosol in the form of inactive procaspase. Caspases need proteolytic cleavage for activation. Apoptosis is crucial for normal tissue physiology, and disturbed ability of apoptosis is responsible for various disorders, such as genetic and autoimmune diseases, neurodegenerative disorders and cancer. Furthermore, apoptosis is an effective process by which a virus can induce cell death, spread progeny and concomitantly reduce inflammatory and immune response (Buenz & Howe, 2006, Everett & McFadden, 2001).

RNA viruses, like the enteroviruses, replicate much more efficiently than large and slow replicating DNA viruses, and their multiplication appears to benefit from rapid initiation of cell death. Enterovirus infection can trigger either canonical cytopathic effect (CPE) or apoptosis, or both, in host cells (Buenz & Howe, 2006). Several studies have proposed anti-apoptotic response from enterovirus infection, counteracting the intrinsic apoptosis program triggered by virus-induced modifications in host cell metabolism (van

Kuppeveld *et al*., 2005). However, full-scale apoptosis seems to be activated only at the end of enterovirus growth cycle after completed viral replication, indicating anti-apoptotic delay rather than blocking of the apoptosis pathway (van Kuppeveld *et al*., 2005). Regardless of the short replication cycle, these viruses are also able to cause persistent infection (Buenz & Howe, 2006), affecting the apoptotic balance and escaping the immune defense. Apoptosis may also be involved in the pathogenesis of enterovirus diseases, such as myocarditis and dilated cardiomyopathy (Carthy *et al*., 1998), as well as neurological diseases (Chen *et al*., 2007).

Enteroviruses have been reported to either favor or inhibit apoptotic events in the cell. EV71 induced apoptosis in neuronal cells caused stimulation of c-Abi activity, which lead to the phosporylation and activation of Cdk5 (Chen *et al*., 2007). CBV3-induced apoptosis has been found to require activation of the MAPK pathway (Luo *et al*., 2002). CBV3 infection is frequently the cause of virus-induced myocarditis, and a critical determinant of the severity of the disease is early onset of apoptosis (Tam, 2006, Yang *et al*., 1999). However, CBV3 infection has also been shown to promote cell survival by degradation of p53 and downregulation of ATF3 (Hwang *et al*., 2007). PI3K/Akt, which has been reported to play an anti-apoptotic role in numerous viral infections (Cooray, 2004), has been reported to have a role in the regulation of apoptosis during CBV3 (Esfandiarei *et al*., 2004), PV (Autret *et al*., 2008) and EV71 (Tung *et al*., 2007, Wong *et al*., 2005) infection. PV is able to prevent tumor necrosis factor (TNF)-triggered apoptosis by abolishing the cytokine receptor from the cell membrane (Neznanov *et al.*, 2001).

Depending of the phase in the PV infection cycle, the virus has been shown to promote either cell survival or apoptosis. In PV-infected cells, the amount of phosphorylated Akt increased until 30 min p.i and then decreased at 4 h p.i. to a level similar to mock infected cells (Autret *et al*., 2008), and later (6h p.i.) apotosis was triggered by a c-Jun NH2-terminal kinase (JNK) reaction cascade (Autret *et al*., 2007).

2.7 TYPE 1 DIABETES

Type 1 diabetes (T1D) accounts for 5-10% of all the cases of diabetes. It is characterized by progressive destruction of insulin-producing β-cells in the pancreatic islets (Devendra *et al*., 2004). The destructive process can start years before the first clinical symptoms. Two distinct forms of T1D have been identified: type 1A is caused by cell-mediated autoimmune attack on β-cells (Devendra *et al*., 2004), whereas type 1B is considerably less frequent, has no known cause, is characterized by varying degrees of insulin deficiency as well as by sporadic periods of ketoacidosis (Abiru *et al*., 2002). Susceptibility to T1D is largely inherited, and over 40% of the familial clustering of T1D

can be attributed to genetic variation in the human leukocyte (HLA) region. The direct involvement of HLA class II DRB1, DQA1 and DQB1 genes is well recognized. The most predisposing DRB1-DQB1 haplotypes are DR3 (DRB1*0301, DQA1*0501, DQB1*0201) and DR4 (DRB1*0401/2/4/5, DQA1*0301, DQB1*0302). Of these, the most predisposing are DRB1*04 subtypes (0401 and 0405). In addition, other allelic variation affecting susceptibility for T1D has been found in classical HLA loci, in class1 and DPB1, and in recent research the region of MHC class III has been of particular interest (Valdes *et al*., 2010). Clinical T1D starts to develop when one or more environmental triggers alter the immune function to initiate β-cell destruction. Several different environmental factors are thought to have a role in this process. These include viruses (e.g. enteroviruses, rubella virus, endogenous retroviruses and human cytomegalovirus) (Lammi *et al*., 2005), bacterial toxin (bafilomycin A1) (Myers *et al*., 2001, Myers *et al*., 2002), environmental toxins (e.g. nitrosamines) (Helgason & Jonasson, 1981), nutrition (e.g. early exposure to cow's milk proteins, cereals or gluten) (Akerblom *et al*., 2002, Norris *et al*., 2003, Thorsdottir & Ramel, 2003, Vaarala *et al*., 1999, Virtanen *et al*., 2000), overweight (earlier onset of T1D in obese children) (Betts *et al*., 2005) and increased hygiene (atopic disorders and T1D are more common in affluent societies than in traditional societies with a lower degree of hygiene) (Beyan *et al*., 2003, Kaila & Taback, 2001).

The pathogenesis of T1D is associated with inflammatory response with infiltration of lymphocytes in the islet of Langerhans and production of autoantibodies against β-cell antigens. There are three autoantibodies that have been shown to predict clinical T1D. These include insulin autoantibodies (IAA), autoantibodies to the 65 kD isoform of glutamic acid decarboxylase (GADA), and the phosphatase-related islet antigen 2 (IA-2). In addition, recently an additional autoantigen in T1D, a zinc transporter 8 (ZnT8), was detected (Wenzlau *et al*., 2007).

The most important group of viruses that has been associated with type 1 diabetes (T1D) are enteroviruses (Barrett-Connor, 1985, Hyöty & Taylor, 2002, Roivainen *et al*., 1998). They replicate in various target organs including the pancreas (Kaplan *et al*., 1983). Association with T1D and enterovirus infections has been reported in prediabetic children (Hiltunen *et al*., 1997, Hyöty *et al*., 1995), adults (Andreoletti *et al*., 1997, Gamble *et al*., 1969), and infections during pregnancy may contribute to early onset of T1D in childhood (Dahlquist *et al*., 1995). Increased prevalence of enterovirus RNA and higher antibody levels against CBVs have been reported in individuals with recent onset of T1D (Andreoletti *et al*., 1997, Gamble *et al*., 1969, Lönnrot *et al*., 2000). In a recent study, immunochemistry staining with enterovirus capsid protein vp1 antibody of autopsy samples showed increased enterovirus prevalence in the islets of patients with recent-onset T1D diabetes (Richardson *et al*., 2009).

Several enteroviruses have been suggested to have a role in the onset of T1D. CBVs, especially serotype CBV4, CAV9 and several serotypes of EVs, such as EV3, 6, 9, 16, 21

and 30, have been associated with T1D (Elshebani *et al*., 2007, Roivainen, 2006, Williams *et al*., 2006). In fact, all enteroviruses that have been investigated in islet cell culture or in animals studies contain strains that are able to damage β-cells (Roivainen & Klingel, 2009). In a non-obese diabetic (NOD) mouse study, only a minority of different CBV4 strains were able to induce diabetes (Yoon *et al*., 1986). However, there is evidence that some enteroviruses, like CBVs, are less prevalent in countries with high incidence of T1D, e.g. Finland, than in genetically similar populations with a low incidence of T1D (e.g. Russian Karelia) (Viskari *et al*., 2005). Frequency of enterovirus infections has decreased during the 1980s and 1990s, while the incidence of type 1 diabetes has increased (Viskari *et al*., 2004), suggesting an inverse relation between enterovirus infection and frequency of T1D. In addition, pregnant women in Finland had lower enterovirus antibody levels than those in Estonia and Karelia (Viskari *et al*., 2005). The neutralizing antibodies from the mother protect the child from infection during the first 3 to 9 months of age. Enterovirus infection during 6-12 months of age has been shown to decrease the risk of T1D (Blom *et al*., 1991, Gibbon *et al*., 1997, Pundziute-Lycka *et al*., 2000), while severe neonatal infections are a risk factor for diabetes (Anonymous, 2000, Dahlquist *et al*., 1999). Similar results have been obtained in a study with NOD mice, where early (pre-insulitis) caused by CBV infection, protected against T1D, while later infection after development of insulitis could accelerate the onset of diabetes (Serreze *et al*., 2005).

The mechanisms by which enteroviruses are able to affect T1D pathogenesis are still largely unsolved, but evidence supporting a few different alternatives exist, including direct destruction of islets, molecular mimicry, bystander destruction and viral persistence (Richer & Horwitz, 2009).

Enterovirus infection both in prediabetic individuals and individuals with newly diagnosed T1D has been associated with antibodies against T1D-related antigens, such as glutamic acid decarboxylase (GAD65) (Baekkeskov *et al*., 1990), insulin (Palmer, 1987), tyrosine phospatases IA-2 and IA-2beta (Notkins *et al*., 1998) and heat shock protein 60/65 (Birk *et al*., 1996, Elias *et al*., 1990). In a study by Elshebani et al., isolated enteroviruses replicated in human β-cells and caused destruction of a share of the cells. However, insulin secretion was reduced in all infected cells, suggesting that these effects on β-cells could lead to cellular stress, possibly triggering mechanisms that could affect the development of T1D (Elshebani *et al*., 2007). It has been speculated that enteroviruses cause infection leading to engulfment of the infected islets and presentation of β-cell antigens in an inflammatory process, a mechanism that has been referred to as bystander destruction (Horwitz *et al*., 1998). The contribution of the bystander mechanism is supported by a study where patients with newly diagnosed T1D were found to have increased levels of IFN-α and half of these patients were concomitantly positive for enterovirus RNA (Chehadeh *et al*., 2000). Furthermore, local infection in non-islet cells of the pancreas might cause inflammation and cytokine-mediated β-cell damage and

destruction of β-cells, resulting in exposure of previously hidden autoantigens and stimulation of autoreactive T cells, a process referred to as the ‘’innocent bystander ‘’ mechanism (Horwitz *et al*., 1998). CBV4 and probably most of the enteroviruses can trigger production of pro-inflammatory cytokines, such as IL-1β, TNF-α, IL-2 and the type 1 interferons (Vreugdenhil *et al*., 2000).

In addition, enterovirus infection in any tissue might induce antiviral responses that cross-react with autoantigens. This could lead to decreased tolerance to certain antigens and autoimmunity (Atkinson *et al*., 1994, Kaufman *et al*., 1993, Vreugdenhil *et al*., 1998). The cross-reactivity could arise from molecular mimicry, where similar structures are shared between viral and host molecules (Maclaren & Atkinson, 1997, Oldstone, 1998). Possible immunological cross-reactivity between enteroviral proteins (2C, VP1 and VP0) and β-cell autoantigens (GAD-65, IA-2/IA-2beta and HSP-60) has been documented (Härkönen *et al*., 2002, Härkönen *et al*., 2003, Varela-Calvino & Peakman, 2003)**.** It has been suggested that CBV4 infection could lead to activation of T lymphocytes that cross-react with pancreatic β-cells. In studies with CBV4, cross-reactivity has been detected between homologous regions of the virus antigens and GAD65 and IA-2/IA-2beta (Härkönen *et al*., 2002, Kaufman *et al*., 1992). A conserved region of the VP1 region of the virus was found to cross-react with IA-2/IA-2beta (Härkönen *et al*., 2002). GAD65 was found to share a sequence homology with CBV4 protein and 2C protein (Kaufman *et al*., 1992). GAD65 have been shown to be able to activate Th1 cells to secrete IFN-γ, which seems to have a role in the pathogenesis of T1D (Karlsson & Ludvigsson, 1998). However, this hypothesis has been criticized because of the fact that T-cells do not cross-react with GAD in vitro (Denman & Rager-Zisman, 2004, Roep *et al*., 2002). The role of GAD65 for T1D development has been tested in vivo. In a study with GAD65-deficient NOD mice, the cumulative incidence of autoimmune diabetes was not influenced by this gene (Atkinson & Leiter, 1999). Moreover, CBV infections were found to be frequently associated with immune reactions against GAD, but the reactions were transient (Cainelli *et al*., 2000). This suggests that other environmental factors are included in the development of clinical disease. IA-2 autoantibodies have also been detected in patients after enterovirus infection, e.g. in a case of EV9 infection and acute onset of T1D (Vreugdenhil *et al*., 2000), and in a prediabetic individual, EV3 infection induced prolonged secretion of this autoantibody (Williams *et al*., 2006). VP1 of CAV9 has shown cross-reactivity with HSP60 (Härkönen *et al*., 2003). However, mimicry is very common in nature, suggesting that the cause is not the phenomenon as such, but rather the crucial factor might be impaired immune regulation.

Fulminant type 1 diabetes, a subtype of type 1B diabetes is characterized by abrupt destruction of β-cells and onset of acute hyperglycemia and ketoacidosis (Imagawa *et al*., 2000, Tanaka *et al*., 2000). A recent report by Tanaka et al. suggested a role for enteroviruses in fulminant type 1 diabetes in three affected pancreata. They implicated that

expression of interferon-γ and CXC chemokine ligand 10 (CXCL10) are initiated in the β-cells of enterovirus-infected pancreas (Tanaka *et al*., 2009). This was in line with a former study showing that enterovirus infection of pancreatic β-cells induced CXCL10 expression within 1-2 days after infection (Berg *et al*., 2006, Hultcrantz *et al*., 2007). CXCL10, secreted from the β-cells, activates and enhances infiltration of T-cells and macrophages to the islet cells. These cells then release inflammatory cytokines, including interferon-γ, simultaneously damaging the islets and further increasing CXCL10 secretion, leading to accelerated cell-mediated autoimmunity and complete β-cell destruction (Tanaka *et al*., 2009). These results were further supported by the lack of induction of CXCL10 or interferon-γ in pancreatic β-cells from slowly developing T1D (Tanaka *et al*., 2009).

3 AIMS OF THE STUDY

Integrins are a family of transmembrane glycoproteins that interact with the ECM in cell adhesion and migration. Altered expression of integrins in tumor cells contributes to metastasis tendency by influencing attachment to adjacent cells, migration and survival. In addition to natural ligands (such as vitronectin and collagen), viral pathogens including certain enteroviruses utilize integrins to enter host cells. Enteroviruses cause a wide spectrum of illnesses varying from common cold to myocarditis and paralysis. Several studies have suggested that enteroviruses could also be involved in the etiopathogenesis of type 1 diabetes.

SPECIFIC AIMS:

1. The role of integrins in the pathogenesis of metastasis to cortical bone

- To study if certain integrins play a role in the initial attachment of mammary carcinoma cells to bone.
- To study if collagen type 1 would be important in this attachment of tumor cells to the skeletal tissue.
- To determine whether the type and amount of integrins expressed on the cell membrane of various tumors correlates with their capability to attach to cortical bone.

2. The relevance of polymorphism in enterovirus receptors in type 1 diabetes

- To study if susceptibility to enterovirus infections and/or frequency of the occurrence of enterovirus antibodies in prediabetic and diabetic children would correlate with variation in the enterovirus receptor genes.

3. Altered targeting of echovirus 1 from the lipid raft/caveolae internalization route to the clathrin pathway

- To produce a chimeric molecular construct, in which the α2I domain of α2β1 integrin was inserted into the extracellular terminus of the transferrin receptor, which is endocytosed via the clathrin-dependent route instead of the lipid raft/caveolae internalization pathway originally used by the virus.
- To determine whether the altered receptor and entry route permits successful internalization and initiation of EV1 infection.

4. Cellular gene expression during EV1 infection

- To investigate the cellular gene expression profile induced by EV1 infection with specific emphasis on immediate early genes.

4 MATERIALS AND METHODS

4.1 Viruses (II, III)

Coxsackievirus A9 (CAV9), coxsackievirus A18 (CAV18), coxsackievirus A21 (CAV21), coxsackievirus B (CBV) 1-6, echovirus 1 (EV1), echovirus 6 (EV6), echovirus 7 (EV7), echovirus 11 (EV11), echovirus 30 (EV30), human rhinovirus 2 (HRV2), human rhinovirus 14 (HRV14) and human parechovirus 1 (HPV1) were originally obtained from the American Type Culture Collection (ATCC). The poliovirus strains used were the Sabin vaccine strains 1-3 (ATCC). These viruses were used in the neutralization assays (see below). EV1 (Farouk strain; ATCC) was propagated in green monkey kidney (GMK) cells, the infected cells and the supernatant were freeze-thawed three times and the virus was precipitated by PEG/NaCl and purified by ultracentrifugation in 5-20% sucrose gradients (Abraham & Colonno, 1984). The infectivities of the viruses were determined by plaque titration.

4.2 Antibodies and other reagents (I, III)

The following antibodies were used: rabbit antiserum and mouse monoclonal antibody (MAb) against caveolin-1 (Transduction Laboratories); anti-CD49b MAb (Immunotech), A211E10, recognizing the I domain of the α2 integrin (a kind gift from Fedor Berditchevski, Institute of Cancer studies, Birmingham, UK); blocking MAb antibodies against α2 (P1E6), α3 (P1B5), α4 (P4G9), and α5 (P1D6) and β1 (P4C10) subunits of integrin receptors (GIBCO); mouse anti-human integrin αvβ3 monoclonal antibody (Chemicon); rabbit anti-bovine collagen type I polyclonal antiserum (Chemicon); rabbit antiserum against Rab11 (Zymed); rabbit antiserum against Rab4 (Abcam); rabbit antiserum against mannose 6 phosphate receptor (CI-MPR) (Marjomäki *et al*. 1990); MAb against EEA1 (Transduction Laboratories); MAb PDI (1D3; Stressgen) a rat monoclonal antibody (1D4B) against Lamp1 (the Development Hybridoma Bank, University of Iowa), mouse anti-GM130 (BD Transduction Laboratories), mouse anti-TfR (Zymed); rabbit antiserum against transferrin (Dakopatts); rabbit antiserum against the late endosome marker 6C4 (a kind gift from Jean Gruenberg, Department of Biochemistry, Geneva, Switzerland); and rabbit antiserum against purified EV1 (Marjomäki *et al*. 2002), adsorbed on SAOS-α2β1 cells to reduce the background and subsequently filtered. Alexa Fluor (AF) 488-conjugated goat anti-rat IgG, AF488- and AF555-conjugated goat anti-mouse IgG and anti-rabbit IgG (Molecular Probes), as well as PE–conjugated rat anti-mouse IgG (BD Transduction Laboratories) were used as secondary antibodies. Human transferrin (Tf) (Sigma), AF488- and AF568-conjugated Tf

(Molecular Probes) were also used. Blocking RGDS peptide was from GIBCO. Collagenase type I, Chlorpromazine hydrochloride and nocodazole were purchased from Sigma.

4.3 Cell cultures (I-III) and transfections (III)

Several different cell lines were used in the studies, including human cervical cancer cells (HeLa), human lung carcinoma A-549 cells, green monkey kidney (GMK) cells, mouse fibroblast cells (NIH3T3), Chinese hamster ovary (CHO) cells, baby hamster kidney cells (BHK-21), human osteosarcoma cells (SAOS), human breast cancer cells (MDA-MB-231), human neuroblastoma cells (SH-SY5Y), human T-cell leukemia cell line jurkat, rhabdomyosarcoma (RD) cells and prostate cancer cells (PC-3). All these cells were originally obtained from the ATCC. The SAOS-α2β1 cell line was obtained by stable transfection of the SAOS cells, with expression of a construct encoding the α2 integrin (Ivaska et al., 1999). NIH3T3, CHO, BHK-21, SAOS, SAOS-α2β1, MDA-MB-231, SH-SY5Y, RD and PC-3 cells were grown in DMEM. GMK cells were propagated in MEM medium. A549 cells were cultured in F12 medium. Jurkat and Hela cells were grown in RPMI-1640 culture medium. The different culture mediums were supplemeted with 10% fetal calf serum (FCS), 100 IU/ml penicillin, and 100 μg/ml streptomycin. The SAOS-α2β1 cells were cultured in the presence of a selection antibody (200 μg/ml of G418; Gibco). Transient transfections with the constructs pcDNA/Zeo3.1-TfR-α2I and -TfR were performed in subconfluent NIH3T3, BHK-21 and CHO cells with Lipofectamine 2000 (Invitrogene) according to the manufacturer's instructions.

4.4 RNA and DNA purification (II)

Confluent A-549 cells, infected with 20 MOI of EV1, were collected after 30 min, 2 h, 4 h, 6 h and 10 h. Total cellular RNA was in a guanidinium lysis buffer and frozen at -70°C. Total RNA was purified by ultracentrifugation thiocyanate-CsCl method (Chirgwin *et al*., 1979). Furthermore, the RNA was DNase (Sigma) treated according to the manufactures instructions. Alternatively, total RNA was purified according to the manufacturer's instructions using RNeasy Mini kit (Qiagen) supplemented with QIAshredder (Qiagen) for homogenization of cells and RNase-FreeDNase Set (Qiagen) to remove DNA completely during RNA purification.

DNA was isolated from peripheral blood mononuclear cells (PBMCs) by two methods; NucleoSpin blood kit (Macherey-Nagel), which was used according to the manufacturer's instructions, and a non-enzymatic method for purification of DNA from blood (Lahiri &

Nurnberger, 1991). In the latter method, PBMCs were lysed with Nonidet P-40 (Sigma) and centrifugated. The protein was separated from the nuclear pellet by incubation with sodium dodecyl sulfate (SDS) and NaCl precipitation. After centrifugation the DNA from the supernatant was extracted by ethanol precipitation.

4.5 Design of primers, construction of the chimeric receptor and sequencing (II, III)

Oligonucletide primers for polymerase chain reaction (PCR) were selected from the region of receptor sequence coding for domains shown to be crucial for enterovirus binding (II, Fig 1, Table 1). The oligonucleotide primers for PVR were from exon 2. The ICAM-1 primers, amplifying receptors for the major group of HRV and some CAVs were from exon 2. The oligonucleotides for CAR were from exons 2 to 5. The oligonucleotide primer for the two integrins, α2β1 and αvβ3, were selected from exon 7 of integrin alpha 2 I-domain and exon 4 of the β3 subunit. The DAF oligonucleotides spanned the SCR2, SCR3a and SCR3b regions.

For construction of a chimeric receptor consisting of TfR and a2I, oligonucletide primers were designed for the a2I and the complete TfR. For the cloning procedure, BamHI restriction site was added to the 5' end and NotI site to the 3'end of the TfR sequence, and NotI and XbaI sites to the 5'and 3' ends of the α2I sequence.

For amplification of PVR, ICAM-1, DAF, CAR, integrin subunits α2 and β3, DNA purified from PBMCs was used as template for the generation of the PCR products by AmpliTaq Gold DNA-polymerase (Perkin-Elmer). pAWneo2-α2 construct (Riikonen *et al*., 1995), containing the complete human integrin α2 subunit and pSFV1-TfR vector (Liljestrom & Garoff, 1991) with the TfR gene were used as templates for the generation of the PCR products by Phusion High-Fidelity DNA Polymerase (Finnzymes). A-overhangs were added to the 3' end of the PCR products by DyNAzyme II DNA Polymerase (Finnzymes). The reactions were run in a thermal cycler (Perkin-Elmer Cetus). The amplicons were purified using the Qiaex II Gel Extraction Kit (Qiagen). The PCR products were first cloned in the pGEM-T Easy Vector System I (Promega) and then assembled in a mammalian cell expression vector pcDNA/Zeo3.1+ (Invitrogen) resulting in TfR- and α2I-TfR-containing constructs (III, Fig 1). The PCR amplicons and the TfR- and α2I-TfR-containing constructs were sequenced with fluorescent dye-labeled terminators using an automated DNA Sequencer (Applied Biosystems) according to the manufacturer's instructions. Primers used for PCR reactions were used in the sequencing reactions. Nucleotide sequences were analyzed with Wisconsin Package Version 10.0, Genetic Computer Group (GCG; Madison, WI).

4.6 RT-PCR (III)

NIH3T3 cells, which were grown on 24 well plates and transiently transfected with the TfR or TfR-α2I constructs, were infected with EV1 (1 h, 4°C) and then incubated for different time periods (0 to 24 h) at 37 °C. The cells were harvested and stored at -80 °C until tested. The cell pellet was suspended in PBS and subjected to automated nucleic acid extraction (Nuclisens easyMag, BioMerieux) with an elution volume of 55 µl. RNA (10 µl) was reverse-transcribed in two separate reactions with either antisense (4-) or sense (3+) primer from the 5'-noncoding region of picornaviruses as described earlier (Santti *et al*., 1997). EV1 RNA from purified virions with spectrofotometrically determined copy number was used as the quantitative standard. PCR was performed in a RotorGene 3000 instrument (Corbett Life Sciences) in 25 µl reactions containing QuantiTect SYBR Green PCR mix (Qiagen, Hilden), 600 nM of 4+ and 3- primers, and 5 µl of the RT-reaction product. Amplification steps were: 95°C for 15 min followed by 45 cycles at 95°C for 15 min, at 65–55°C for 30 s (touch-down 1°C/cycle for first 10 cycles) and at 72°C for 40 s.

4.7 HLA typing (II)

The informative HLA-DQB1-DQA1 and DRB1 alleles as genetic risk factors for T1D were defined using a typing method based on PCR amplicification of the gene segments and hybridization with a panel of lanthanide-labeled sequence-specific oligonucleotide probes (Nejentsev *et al*., 1999).

4.8 Cell adhesion assay measuring attachment to cortical bone (I)

Bovine cortical long bones were obtained from a local slaughterhouse and stored at -20 °C. The femoral cortical shaft was cleaned from soft tissue and bone marrow. The bone was cut and shaped on a lathe to round rods with a diameter of 6 mm, which were further cut by a diamond wafering saw blade into 200 µm thin disks. The bone slices were placed into 96 well microplates (Greiner GmbH, Frickenhausen) and treated with 1% bovine serum albumin (BSA) in RPMI 1640 medium for 15 min at RT. MDA-MB-231 cells were loaded with acetoxy methyl ester of 2', 7'-bis-(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF-AM) (Molecular Probes) for 15 min at 37 °C in RPMI 1640 medium before adding to bone disks. The plates were then centrifuged for 3 min at 800 rpm. The cells were washed with a Na^+-solution (140 mM NaCl, 1mM $CaCl_2$, and 20 mM Hepes (pH 7,2) and re-suspended in K^+-solution (pH 8), 140 mM KCl, 1mM $CaCl_2$, and 20 mM

Hepes (pH 7,2) containing 10 µM nigericin (Sigma). The fluorescence from cells attached to bone was measured in the Fluoroscan II microplate fluorometer (Labsystem).

4.9 FACS analysis (I, III)

MDA-MB-231 and CHO cells, transfected with pcDNA/Zeo3.1-TfR and pcDNA/Zeo3.1-TfR-α2I constructs were detached with EDTA. The MDA-MB-231 cells were incubated for 45 min at 4°C in 1:100 dilutions with blocking MAb antibodies against α2 (P1E6), α3 (P1B5), α4 (P4G9), α5 (P1D6) and β1 (P4C10) subunits of integrin receptors (GIBCO), and integrin αvβ3 (Chemicon). The cells were washed with PBS and treated with FITC conjugated rabbit anti-mouse Ig (Boehringer Mannheim) for 45 min at 4°C. CHO cells, transfected with the pcDNA/Zeo3.1-TfR or pcDNA/Zeo3.1-TfR-α2I constructs, were incubated for 1 h with 100 µg/ml of Tf, washed with PBS and incubated with MAb against Tf (Dakopatts) and α2I (A211E10). AF488-conjugated goatanti-rabbit and PE-conjugated ratanti-mouse were used as secondary antibodies. The transfected CHO cells were fixed with 4 % formaldehyde for 20 min. Fluorescence intensity was measured by FACSCalibur flow cytometer (BD Biosciences, San Jose, CA) and CellQuest Pro software (BD Biosciences, San Jose, CA). Raw data was further analyzed with WinMDI 2.9 software (J. Trotter, Scripps Research Institute, La Jolla, CA). The MDA-MB-231 cells were analyzed on FACScan cytometer (Becton Dickson, Mountain View, CA, USA).

4.10 Immunofluorescence and confocal microscopy (III)

During a 1h incubation period at 4 °C EV1 was allowed to bind to the NIH3T3, BHK-21 or CHO cells transfected with pcDNA/Zeo3.1-TfR or pcDNA/Zeo3.1-TfR-α2I constructs. Unbound virus was removed from the cells before further incubation at 37 °C. Human holo-Tf, AF488- or AF568-conjugated Tf was added to virus- and mock-infected NIH3T3 cells transfected with pcDNA/Zeo3.1-TfR or pcDNA/Zeo3.1-TfR-α2I constructs and incubated at 37 °C. Alternatively, the transfected NIH3T3 cells were incubated for 15 min in the presence of holo-Tf, AF488- or AF568-conjugated Tf prior to infection. The cells were fixed with 4 % formaldehyde (Sigma) for 20 min, quenched with 50 mM NH_4Cl for 10 min and permeabilized with 0.3 % Triton-X 100 (BDH) in PBS if not otherwise mentioned and stained with primary and secondary antibodies. The cells were studied with an axiovert confocal microscope Leica TCS SP2 with HCX PL APO 63x/1.32-0.6 oil objective or with Olympus Fluoview 1000 confocal setup. Images were acquired using Fluotar 60x objective (60 x 1.25, oil) with digital resolution of 512 x 512. False

colocalization signals were avoided by scanning fluorescence from different excitation wavelengths separately. The images were analyzed with Leica (LCS-Lite) or Olympus (FV10-ASW 1.4) software and then converted further to JPEG or TIFF format for additional processing in Photoshop (Adobe systems). For quantitative confocal microscopy, samples of EV1 infected NIH3T3 cells, transfected with the pcDNA/Zeo3.1-TfR-α2I construct, were collected at different time points and immunostained with antibodies against EV1 and α2I, followed by secondary antibodies (AF488-conjugated goat anti-mouse and AF555-conjugated goat anti-rabbit). The samples were examined by confocal microscopy, and Z-section images were taken from the upper surface to the bottom of each cell. Colocalization of α2I and EV1 in 15 to 20 cells from each time point was analysed with BioimageXD (2007).

4.11 Infectivity assays with drugs (III)

SAOS- α2 and NIH3T3 cells, transfected with pcDNA/Zeo3.1-TfR or pcDNA/Zeo3.1-TfR-α2I constructs, were preincubated in 33μM chlorpromazine hydrochloride (Sigma) or 25 μM nocodazole (Sigma) for 30 min at 37 °C before addition of EV1 for 1 h at 4 °C. Unbound virus was removed and the cells were further incubated at 37 °C for 2min to 6h. After fixation, the cells were stained with antibodies against EV1, α2I and EE1, and the virus localization in the cell was studied with confocal microscopy.

4.12 Determination of neutralizing antibody levels (II)

Serum specimens were diluted serially at 2-fold steps (1:8-65 536) on microtiter plates (Nunclon Microtest plates, 96-well). Pretitrated virus solution corresponding to 100 TCID50 units was added to each well. The viruses that were used included CAV-9,-18, -21, CBV1-6, EV-1, -6, -7, -11, HRV-1,-14, and HPV1, all obtained from ATTC, and PV were Sabin vaccine strains. The mixtures were incubated for 2h at 36 °C and left overnight at RT. 30 000 cells were added to each well. GMK cells were used for CAV9, CBV1-6, PV1-3 and EV11. HeLa cells were used for HRV-2 and -14 and CAV-18 and -21, while A-549 cells were used for EV-1,-6-7, -30 and HPV1. The plates were incubated at 36°C for 6 days before staining with crystal violet.

4.13 Infectivity titration (III)

For the determination of the viral replication cycle, the NIH3T3 cells transfected with pcDNA/Zeo3.1, pcDNA/Zeo3.1-TfR or pcDNA/Zeo3.1-TfR-α2I constructs, SAOS-α2 cells and A549 cells were infected by EV1 and collected after different time points of infection. SAOS-α2 cells were used as positive controls for the transfected NIH3T3 cells. After three freeze-thaw cycles, the amount of intracellular virus was determined in dilutions of 10^{-1} to 10^{-8} in GMK cells by plaque titration.

4.14 Human cDNA U133A arrays and clustering

The Affymetrix Human Genome U133A Array (Affymetrix, Santa Clara, CA, USA) that contains 22215 human gene cDNA probe sets representing ~ 19,000 genes (*i.e.*, each gene may be represented by more than one probe set), were used to perform gene expression analysis. The arrays were scanned and the fluorescence intensities measured by Microarray Suite 5.0 software (Affymetrix). The data was transferred to DNA-Chip Analyzer software (Affymetrix) for normalization and model-based analysis (Li & Wong, 2001). For each probe set a detection p-value was calculated, and any probe sets with $p < 0.04$ were obtained as "present", showing that the gene transcript was reliably detected (Affymetrix, 2001). Differently expressed genes were identified by Affymetrix dCHIP software (www.dCHIP.org). Alterations exceeding 1.5 were considered significant in genes where either of the values was more than 40. To analyze the networks of protein-protein interactions, the STRING 8.2 database was used (Jensen *et al*., 2009).

5 RESULTS AND DISCUSSION

5.1 THE ROLE OF INTEGRINS IN BREAST CANCER METASTASIS TO THE SKELETON (I)

Breast cancer cells frequently metastasize to the skeleton. Preferential attachment of breast cancer cells to bone-specific molecules may facilitate favored metastasis to the skeleton. A requirement for metastatic spread is the capability of the tumor cell to reorganize the cytoskeleton to increase cellular motility. Integrin-mediated adhesion is crucial for the regulation of tissue integrity and function of mammalian tissue. Integrins have been implicated in the multi-step process of metastasis from breast tumor to bone tissue, including detachment and escape from primary organ, invasion through ECM and survival in circulation, capture to a distant endothelium, extravasation across the endothelium, migration through ECM and establishment of secondary tumor through cross-talk with osteoclast and osteoblast (Peyruchaud, 2007, White & Muller, 2007). This frequently leads to osteolytic (bone destructive) lesion, osteoblastic (bone formation) or mixed lytic and blastic lesion can also occur (Kozlow & Guise, 2005). In this study, the role of integrins in the initial attachment of mammary carcinomas to bone was analyzed by an in vitro microplate attachment assay, measuring the binding of MDA-MB-231 breast cancer cells to bovine cortical bone disks. In addition, the correlation between integrin surface expression and tumor's capability to attach to cortical bone was investigated.

5.1.1 High surface expression of α2β1 and α3β1 integrins on human breast cancer MDA-MB-231 facilitates attachment to cortical bone matrix

The expression of integrin molecules on the human breast cancer cell line MDA-MD-231 was determined by FACS analysis. The majority of the cells strongly reacted with the monoclonal antibodies against β1, α2 and α3 integrin subunits, confirming the presence of α2β1 and α3β1 collagen receptors (I, Fig. 1 and Table 1). In addition, antibodies against the α5 integrin subunit stained the cells, while antibodies directed against the α4 integrin subunit or the αvβ3 integrin gave very weak staining. These results were in line with previous studies showing similar patterns of surface integrin expression on these cells (Boissier *et al*., 1997, Morini *et al*., 2000, van der Pluijm *et al*., 1997).

MDA-MB-231 cells have been shown to be able to rapidly attach to cortical bone disks (Nordström *et al*., 1999). To study the relative contribution of the various integrin receptors in the rapid attachment process, the MDA-MB-231 cells were pretreated with function-blocking monoclonal antibodies against various subunits in the integrins before

use in the attachment assay. Pre-treatment of the cells with monoclonal antibodies against the β1 or α2 integrin subunit inhibited the MDA-MB-231 cell attachment to cortical bone by 75 % and 76 % respectively (I, Fig. 2). Cells pretreated with antibody against the α3 subunit inhibited attachment by 26 %, while antibodies against the α5 inhibited attachment by 8%. Antibodies to α4 did not inhibit, as expected, since α4 was not expressed on the MDA-MB-231 cells. Cells pretreated with an antibody against the αvβ3 integrin (vitronectin receptor) had no inhibitory effect on the cell attachment. In line with this, a tetrapeptide containing the RGD recognition sequence had no inhibitory effect on the binding to cortical bone (data not shown). Collagen type I is the major protein present in cortical bone (~90% of total protein) (Carter & Spengler, 1978). To further analyze the role of this protein in the attachment process, MDA-MB-231 breast cancer cell surface collagen receptors were saturated by preincubation with soluble collagen type I. This reduced the binding of cell to bone matrix by 85%. The blocking of rapid attachment of cancer cells by using inhibiting antibodies against the α2 and β1 integrin subunits or by using soluble collagen type I was very close to the 90 % inhibition obtained by pretreating the cells with a mouse antiserum raised against the MDA-MB-231 cell line (data not shown). The results thus implicate that rapid attachment of MDA-MB-231 breast cancer cells to bone matrix mainly involves the α2β1 and α3β1 collagen receptors and collagen type I. The stronger inhibition of attachment of cancer cells to cortical bone disks by antibodies against the α2β1 integrin, compared with those against the α3β1, is consistent with the reports implicating the integrin α2β1 to be the major cell surface collagen type I receptor (Kirchhofer *et al*., 1990, Vihinen *et al*., 1996).

Furthermore, incubating cortical bone disks with collagenase type 1 for 30 min at 37 °C or polyclonal antiserum against bovine collagen type I also had a blocking effect on the attachment by over 40%. However, the much higher inhibition achieved by incubating cells with α2 and β1 integrin antibodies or soluble collagen type I may be explained by the fact that α2β1 and α3β1 collagen receptors are also able to attach to other bone matrix proteins, such as laminin and fibronectin.

In animal models the collagen/laminin receptor a2β1 and the fibronectin receptor α4β1 have been shown to be responsible for selective metastasis of cancer cells to the skeleton (Chan *et al*., 1991, Matsuura *et al*., 1996). Prostate cancer is another tumor that preferentially metastasizes to the skeleton. The attachment to bone of two pancreatic cancer cell lines LNCAPcol obtained by serial passage on type I collagen and C4-2B was observed to be dependent on the α2β1 integrin and its ability to stimulate RhoC GTPase activity (Hall *et al*., 2006, Hall *et al*., 2008). RhoC GTPase has been found to be associated with the progression of many cancers, including breast, melanoma and pancreas (Clark *et al*., 2000, Suwa *et al*., 1998, van Golen *et al*., 2002). RhoC GTPase is associated with poor prognosis in breast and lung cancers (Ikoma *et al*., 2004, Pille *et al*., 2005).

The mechanism for MDA-MB-231 cells' attachment to cortical bone (van der Pluijm *et al*., 1997), basement membrane (Morini *et al*., 2000) and vitronectin has been investigated in the past (Wong *et al*., 1998). In line with our results α3β1 was reported to be crucial for MDA-MB-231 cells' invasion and migration in in vitro cell migration assay. Furthermore, the expression of the α3β1 integrin was determined to be higher in breast cancer metastases than in primary tumors (Morini *et al*., 2000). In contrast to reports by Pluijm et al., where the cells were kept in contact with bone surface for 3h at 37 ˚C (van der Pluijm *et al*., 1997), we did not see any inhibition with antibodies against the vitronectin receptor αvβ3 integrin. Moreover, Wong et al. reported that the αvβ3 integrin did not support the binding of MDA-MB-231 cells to vitronectin (Wong *et al*., 1998). In their experiments they used a vitronectin cell adhesion assay, where cells were allowed to adhere for 30 min at 37 ˚C and a cell migration assay, where cells where allowed to traverse a vitronectin-coated membrane. In contrast, they suggested the αvβ1 integrin receptor to be crucial for MDA-MB-231 cells' attachment to vitronectin, since the binding was inhibited by both RGD peptide and antibody against β1 (Wong *et al*., 1998). In our experiments the cells were not allowed to be in contact with the bone surface for more than 5 min. Consequently, the short time did not allow the cells to spread and form adhesion sites, and microscopic examination of the attached cells clearly showed that they had a round morphology. However, if the cells were attached to bone surface for longer time, they began to spread and acquired a flattened morphology. During longer attachment periods, the cells themselves might secrete adhesion proteins, such as vitronectin, fibronectin and osteopontin, or upregulate new cell surface receptors. The cell spreading started about 30-45 min after their initial attachment (data not shown). This might explain why our results and the ones published by Wong et al. (Wong *et al*., 1998) are different from those published by Pluijm et al. (van der Pluijm *et al*., 1997).

In conclusion, our results suggest an important role for the α2β1 and α3β1 integrin receptors in the initial anchoring of MDA-MB-231 breast cancer cells to cortical bone matrix.

5.1.2 Correlation between surface expression of α2β1 and α3β1 integrins on tumor cells and their tendency to anchor to cortical bone matrix

To further study the roles of α2β1 and α3β1 collagen receptors in cancer cell attachment to cortical bone, the integrin surface expression on three additional human cell lines that included a prostate cancer cell line PC-3, T-cell leukemia line Jurkat and the neuroblastoma cell line SH-SY5Y were studied. PC-3 cells have been reported to preferentially metastasize to the skeleton in nude mice models (Shevrin *et al*., 1988, Shevrin *et al*., 1991) and easily adhere to cortical bone disks (Nordström *et al*., 1999, van

der Pluijm *et al*., 1996a, van der Pluijm *et al*., 1996b). PC-3 expressed high levels of the α2β1 and α3β1 integrin receptors (I, table 1) and was also able to rapidly attach to cortical bone matrix.

In contrast, the human Jurkat and SH-SY5Y cells, which only modestly attached to cortical bone, showed very low expression of these integrins (I, Table 1, Fig. 4). Surface expression of the α4 integrin on Jurkat cells did not support rapid attachment to bone matrix, although in a former study the α4β1 integrin has been suggested to be one candidate that specifically localizes cancer cells to the skeleton through adhesion to VCAM-1 (Matsuura *et al*., 1996).

Together these results implicate a close connection between α2β1 and α3β1 integrin receptor expression and the ability of different cancer cell types to attach to cortical bone.

5.2 VARIATION IN ENTEROVIRUS RECEPTOR GENES (II)

Enteroviruses are believed to contribute to the pathogenesis of type 1 diabetes (T1D). Higher antibody levels against CBVs and precense of enterovirus RNA have been reported in individuals with recent onset of T1D (Andreoletti *et al*., 1997, Gamble *et al*., 1969) and in prediabetic children (Hiltunen *et al*., 1997, Hyöty *et al*., 1995). Moreover, in autopsy patients with recent-onset T1D showed higher prevalence for vp1 in pancreas compared with a control group (Richardson *et al*., 2009).

It is possible that sensitivity to enterovirus infections might correlate with individual variation in enterovirus receptor genes. To investigate this possibility, sequence analysis of the domains known to be critical for virus binding in the major enterovirus receptors (CAR, DAF, ICAM-1, PVR, and α2- and β3 integrin subunits) were performed from human blood samples collected from healthy individuals and children with type 1 diabetes. In parallel, virus-specific neutralizing antibodies and type 1 diabetes risk-associated HLA-alleles were analyzed.

5.2.1 Sequence analysis of enterovirus receptor genes and HLA types

PCR and sequence analysis were carried out for the enterovirus receptor genes CAR, DAF, ICAM-1, PVR, and α2- and β3 integrin subunits for the regions reported to be important for virus binding. DNA samples were acquired from a group of children with T1D (21 individuals) and a control group of healthy adults (20 individuals). Ten additional samples from diabetic children and five samples of healthy children from Estonia were analyzed to clarify the frequency of the variation found in the PVR gene. These samples were also used as additional controls in serological studies.

A heterozygote single nucleotide polymorphism (SNP) was detected in the V-domain (exon 2) of the PVR at Ala 67(GCG)→Thr (ACG) in 4 individuals of the 31 children with T1D (12.5%), but it was not found in the healthy control group. This variation in the variable domain of PVR is placed in the virus binding region and may thus inhibit cellular binding of all three PV serotypes. Heterozygotic polymorphism of this gene was recently suggested to be a risk factor for the development of vaccine-associated or paralytic poliomyelitis associated with wild-type virus (Kindberg et al., 2009). Moreover, this variation has been found to be more frequent among individuals with the motor neuron disease Amyotrophic lateral sclerosis (ALS) than controls (Saunderson *et al*., 2004). In former studies enteroviruses have been suggested to be implicated in the development of this disease (Berger *et al*., 2000, Woodall & Graham, 2004). This variation in the PVR genome has been shown to reduce the infection rate of PV by 50%, increase resistance to cell lysis (Pavio *et al*., 2000) and reduce apoptosis (Gosselin *et al*., 2003) in human neuroblastoma cells. The delayed course of poliovirus-induced catalysis could therefore be connected to persistent infection in the CNS (Kindberg *et al*., 2009). Persistent infection has been suggested to be a possible mechanism in the development of T1D. This could be due to improper immune response to enterovirus infection, leading to viral persistence and increased pancreatic β-cell damage (Richer & Horwitz, 2009). Persistent CBV4 infection in pancreas has been associated with diabetic-like syndrome in mice (Zhou & Li, 2008), and association of persistent infection in gut mucosa and in pancreas has been reported in T1D (Richardson *et al*., 2009). Although former results indicate no relationship between vaccination and T1D (Hviid *et al*., 2004), one could still speculate that oral polio vaccination (OPV) could be a risk factor for T1D in those individuals possessing this genetic variation. However, since the OPV vaccinations have not been used for over a decade in Finland, this could not be the case for those diabetic children included in this study. Therefore, serum samples from Estonian children who had been vaccinated with live poliovirus vaccine were studied. One of the children had no serum antibodies for any of the PV serotypes and one did not have antibodies for the PV3. These children did not, however, have the SNP found in the first part of the study in diabetic children. Moreover, one could speculate that a possible mechanism in the pathogenesis of T1D for those possessing this variation in PVR could be connected with impaired immune response. In a number of studies, PVR has been shown to serve as an important ligand for cell surface receptors expressed on lymphocytes, monocytes and dendritic cells. PVR attaches to DNAX accessory molecule 1 (DNAM-1;CD226) (Bottino *et al*., 2003, Tahara-Hanaoka *et al*., 2004) and Tactile (CD96) (Fuchs *et al*., 2004, Seth *et al*., 2007), which are expressed on NK cells, $CD8^+$ T cells and $CD4^-$ T cells. DNAM-1 regulates monocyte extravasation via its interaction with PVR expressed at endothelial junctions (Reymond *et al*., 2004). In the tissue monocytes can differentiate into dendritic cells and macrophages to elicit an immune response. In addition a recent study showed a novel interaction between PVR and

a novel immunoreceptor, called T cell immunoreceptor with Ig and ITIM domains (TIGIT) (Boles *et al*., 2009), which is expressed on human follicular B helper T cells (TFH).

A heterozygous SNP was also found in the first Ig-like domain (exon 2) of ICAM-1 at Lys56 (AAG)→Met (ATG) in one individual in both the control and the diabetes group. The individual from the diabetes group also had the heterozygous variation found in PVR. ICAM-1 is a receptor for the major receptor group human rhinovirus (Greve *et al*., 1989, Staunton *et al*., 1989) and has also been shown to be used by coxsackievirus A13, A15, A18, A20 and A21 (Colonno, 1986, Pulli *et al*., 1995, Shafren *et al*., 1997b). The SNP found in this study is in the sequence of the first 159 amino acids (domain 1) of ICAM-1 required for human rhinovirus binding (Staunton *et al*., 1990).

The same variation was earlier reported to be associated with increased risk of cerebral malaria and named ICAM-1^{Kilifi} (Fernandez-Reyes *et al*., 1997). However, a later study by A. E. Fry et al. using larger case groups did not support this association (Fry *et al*., 2008). In a later report ICAM-1^{Kilifi} was shown to reduce immune responsiveness since it decreases the avidity to lymphocyte function-associated antigen-1 (LFA-1) and abolishes binding to soluble fibrinogen (Craig *et al*., 2000). Furthermore, ICAM-1^{Kilifi} was observed to prevent HRV16 binding (Xiao *et al*., 2004), and in crystal structure studies the binding of CVA21 to ICAM-1^{Kilifi} was suggested to possibly be weaker than its binding to ICAM-1 (Xiao *et al*., 2005).

Silent SNP variations, both as homozygotic or heterozygotic, were frequently found in both groups from the α2 integrin I domain subunit at Phe43(TTT)→Phe(TTC), and a silent heterozygous SNP in the glutamine codon 111 (CAA→CAG) from CAR exon 2 was also seen in one individual in the diabetes group.

HLA-DR4-DQ8, which is associated with increased risk of type 1 diabetes, was found in 74% of children with type 1 diabetes and in 10 % of the adult control group. Three diabetic children with the SNP in the PVR gene, who were HLA typed, were all positive for HLA-DR4-DQ8. In general, no significant correlation between neutralizing antibodies and the HLA alleles could be seen. HLA genotypes among cases and controls were expected. HLA-DR4-DQ8 was significantly increased and DR2-DQ6 decreased among the children with type 1 diabetes (data not shown).

Taken together, the study shows individual variation in some genes coding for enterovirus receptors. In the PVR, SNP causing amino acid change in a region critical for efficient binding and infection of PV were detected in the first group of individuals with T1D. Interestingly, three of the diabetic children with the SNP in PVR gene, who were HLA typed, were all positive for HLA-DR4-DQ8. However, its direct connection to T1D should be analyzed with a larger number of individuals to obtain statistically sustainable results. Genetic variation was found in ICAM-1 in one individual in both the diabetic and the control group. In contrast, no variation leading to amino acid change occurred in genes

coding for the CAR and DAF, which are the receptors for coxsackie B viruses and echoviruses, showing most frequent association with T1D. However, since only the virus-binding regions of the receptor sequence were studied, it is still possible that other parts of the receptor molecules (e.g. the ones involved in endocytosis and signaling events) may enclose genetic variation that could influence enterovirus infection.

5.2.2 Serological markers of enterovirus infection

Serum antibody levels against a collection of human enteroviruses and human parechovirus 1 were measured by microneutralization assay from the plasma of 21 children with type 1 diabetes, 20 matched control children, and 20 healthy adults. In addition, serum antibodies against poliovirus 1-3 were studied in a group of 43 OPV-vaccinated children from Estonia to search for seronegative individuals possessing the genetic variation found in PVR.

Several enteroviruses have been suspected to be involved as environmental triggers of T1D. Especially the involvement of CBVs and echoviruses has been implicated in the onset of T1D (Roivainen, 2006). In our study, the levels of neutralizing antibody titers against the enteroviruses were higher in the adults than in the children, except for poliovirus 1-3 and human parechovirus 1. In general, no clear difference was detected between serum antibody levels against most of the enteroviruses between the groups of diabetic and control children. Low levels of serum antibodies against EV 1, 6, 7 and 11 were seen in both adults and children, while the vast majority of the children lacked antibodies against CBV 1-6, and no significant differences between the groups of children were observed. These results can partly be explained by the relatively low level of CBV and echovirus infections during the years 1998-2001 compared with the previous three-year period (Register of the Public Health Institute, Finland). Furthermore, CAV18, A21, and HRV2 and 14 antibodies, studied to correlate the findings to the variation in the ICAM-1 gene, were generally low, and no direct conclusions could be made (data not shown).

Seropositivity for CAV9 was found in only two healthy control children, but high individual antibody levels against CAV9 were observed in six diabetic children. However, the difference was not statistically significant (II, Fig. 3). CAV9 infection has been associated with increased production of islet cell autoantibodies in prediabetic children (Roivainen *et al*., 1998). In in vitro studies, the virus was observed to cause persistent infection in human pancreatic islet cells, occasionally accompanied by minor morphological changes associated with impaired functional properties of insulin-producing β-cells (Roivainen *et al*., 2000).

The levels of neutralizing antibodies against EV30 were significantly lower in the children with newly diagnosed T1D when compared with healthy children (P<0.0003) (II, Fig. 2 and 3). EV30 has been shown to be islet cell destructive in in vitro studies (Paananen *et al*., 2007, Roivainen *et al*., 2002) and to sometimes generate a humoral response to homologous regions in islet cell autoantigenes such as IA-2/IA-2beta and HSP-60 (Härkönen *et al*., 2003). However, in a recent study with a group of 100 diabetic children and non-diabetic controls did not support our finding of lower antibody levels in diabetic children, as no difference in antibody levels against EV30 was observed (Paananen *et al*., 2007). EV30, which binds to the SCR1 domain of DAF, was reduced by 21% in rhabdomyosarcoma (RD) cells (data not shown) with monoclonal antibodies against this domain, while monoclonal antibodies recognizing domains two and three had no effect (data not shown). This incomplete inhibition of virus binding could be explained by a result in a recent report by Ylipaasto et al., suggesting that αvβ3 integrin functions as a cellular receptor for EV30 (Ylipaasto *et al*., 2009).

No significant difference in the levels of neutralization antibodies to PV1-3, induced by the inactivated vaccine used in Finland, was found among patients with T1D and healthy children, although slightly higher levels of antibodies to PV2 were observed in healthy children (P=0-056) (II, Fig. 2 and 3). In both groups, a small group of children had low neutralization antibody levels or no antibodies against PV3. Interestingly, three of these children with T1D carried the heterozygotic SNP in the V-domain region of PVR (II, Fig. 2, Table 2). Of the serum samples obtained from Estonia, one was seronegative for all three poliovirus serotypes and one lacked antibodies against PV3. However, the samples in question did not contain the SNP in PVR found in the first group of diabetic children.

In conclusion, the results showed no remarkable difference in the levels of neutralizing antibodies against enteroviruses and rhinoviruses among the groups studied. Higher antibody levels against CAV9 were observed in the T1D group, although the difference was not statistically significant. Interestingly, the level of neutralization antibody against EV30 was significantly lower in the group of diabetic children compared with the group of healthy children.

5.3 ALTERED TARGETING OF EV1 TO CLATHRIN-DEPENDENT PATHWAY (III)

Some picornavirus serotypes, including CBV3, FMDV and EV6, have been shown to be able to use both lipid raft/caveolin- and clathrin-dependent host cell endocytosis, depending on cell type or genetic differences in the virus genome (Chung *et al*., 2005, Coyne *et al*., 2007b, Leveque *et al*., 2007, Wang *et al*., 1993). Echovirus (EV1) has been reported to enter host cells from lipid rafts, through caveolae (Marjomäki *et al*., 2002,

Pietiäinen *et al*., 2004) or by macropinocytosis (Karjalainen *et al*., 2008). The aim of the study was to investigate if EV1 could be directed to the clathrin-dependent entry pathway by a chimeric receptor and would be able to initiate productive infection in host cell.

5.3.1 Colocalization of EV1 and α2I with human transferrin in cells expressing a chimeric TfR-α2I receptor

In order to study whether attachment of EV1 to a receptor mediating clathrin-dependent pathway could lead to successful internalization of the virus, a chimeric molecule was constructed: The α2I domain from human α2 integrin subunit, known to bind EV1, was inserted into the extracellular terminus of human TfR, which normally transports transferrin-bound iron through clathrin-dependent endocytosis (III, Fig. 2). Some animal parvoviruses, including CPV, use TfR as a receptor in clathrin-directed endocytosis (Hueffer *et al*., 2003, Palermo *et al*., 2003). The chimeric TfR-α2I and human TfR were transiently expressed in NIH3T3 and CHO cells. Normally, EV1 does not bind to mouse NIH3T3 cells or hamster CHO cells due to dissimilarities between mouse, hamster and human α2 integrin sequences.

The expression of the receptors in transiently transfected NIH3T3 and CHO cells was analyzed by IF microscopy and flow cytometry (FACS). In IF microscopy using antibodies against α2I and fluorescent Tf, the receptors were seen to be expressed in both cell lines. In flow cytometry (FACS) analysis, CHO cells were first incubated with human Tf at 4 °C, and the receptors were detected by antibodies against α2I and Tf. Both TfR- and TfR-α2I-transfected cells bound human Tf (III, Fig. 2A and B). In addition, nontransfected cells also bound Tf, but more efficient binding was seen in the transfected cell lines (data not shown). Double-labeled cells showed that the same population of TfR-α2I transfected cells was positive for both Tf and α2I (III, Fig. 2 C). This data indicated that the chimeric receptor can function as a receptor for Tf.

To identify whether EV1 could enter by clathrin-directed endocytosis into NIH3T3 cells, transiently transfected with the TfR-α2I and TfR receptor constructs, colocalization between Tf, α2I and EV1 was analyzed by confocal microscopy (III, Fig. 3). EV1 was first attached to the cells for 1 h at 4°C (indicated as 0 h p.i.). Then the cells were incubated with Tf at 37°C or, alternatively, the cells were first incubated with Tf for 15 min at 37°C before attaching of virus to the cells. Colocalization between Tf and α2I (III, Fig. 3A) and partial colocalization of Tf and EV1 was seen from 15 min (III, Fig. 3 B) to 2 h p.i.

These results showed that both the TfR and the chimeric receptor are functional Tf receptors and the chimeric construct can act as an internalization receptor for EV1. In addition, these results indicate that EV1 is endocytosed through a clathrin-directed pathway and colocalizes with Tf in recycling endosomes.

5.3.2 EV1 attaches and enters host cells expressing TfR-α2I chimeric receptor

To further study the interaction between EV1 with the TfR-α2I chimeric receptor, EV1 was attached to TfR-α2I-transfected NIH3T3 cells. The virus and the receptor were detected by antibodies against α2I and EV1 and studied by confocal microscopy. EV1 bound to the TfR-α2I chimeric receptor on the cell surface, and 30 min p.i., colocalization between α2I and EV1 in vesicular structures was detected near the cell membrane (III, Fig. 4A). EV1 mainly remained in intracellular structures 2 h p.i., while the receptor was recycled to the plasma membrane, typical for TfR. This differs from canine parvovirus (CPV) entry, where the virus remains attached to TFR for several hours (Parker *et al*., 2001). EV1 could not attach to or enter NIH3T3 cells, transfected with TfR (III, Fig. 4B). Furthermore, the colocalization of the virus and chimeric receptor was studied by quantitative confocal microscopy. Z-sections were obtained from EV1-infected NIH3T3 cells, transfected with the chimeric receptor and analysed with BioimageXD software. The colocalization between α2I and EV1 diminished during a time period spanning from 0 h p.i. to 30 min p.i., indicating gradual detaching of the virus from the receptor.

5.3.3 Colocalization of EV1 and the chimera receptor with cellular markers

Clathrin-mediated transport is crucial for several cellular processes, including nutrient uptake, uptake of iron-loaded transferrin that binds to TfR, synaptic vesicle recycling and selective endocytosis of receptor-bound ligands (Conner & Schmid, 2003, Simonsen *et al*., 2001). Receptor-bound ligands are transported from clathrin-coated vesicles to early endosomes (EEs), which possess a slightly acidic milieu, causing dissociation of some viruses from the receptor and release of the genome to cytoplasm (Mukherjee *et al*., 1999). The endocytic pathway of EV1 in TfR-α2I-transfected cells was studied using different antibodies against intracellular organelle markers in confocal microscopy. Cellular markers for EE included the early endosome antigen 1 (EEA-1) and Rab4, a small GTPase. EV1- infected BHK-21 cells, double-stained for EV1 and EEA-1, showed partial colocalization 2 min p.i. (III, Fig. 5A) that disappeared already 5 min p.i. The BHK-21 cells that are normally not infected by EV1 were used because of a stronger fluorescence signal of EEA-1 compared with the NIH3T3 cells. In EV1-infected, TfR-α2I-transfected NIH3T3 cells, clear colocalization between α2I and Rab4 was seen 2 min p.i. (III, Fig. 5B).

The cargo from EE can be transported to RE. The iron-Tf-TfR complex is rapidly endocytosed into clathrin-coated pits (Mellman, 1996) and further transported to early or sorting endosome. In the endosomal acidic mileu, iron is released from Tf and the resultant apo-Tf-TfR is recycled to the plasma membrane by fast endosome-directed

transport dependent on Rab4 or through a slower process via perinuclear recycling compartments, dependent on Rab11 (Hao & Maxfield, 2000, Morgan, 1996, Qian *et al*., 1997, Sheff *et al*., 1999). Furthermore, the ligand-receptor complex can be transported from EE to LE, possessing a more acidic milieu for instance during endocytosis of the CPV-TfR complex (Parker *et al*., 2001). Alternatively, CPV is transported from EE shortly after cell entry to perinuclar recycling endosomes (PNRE) where the virus has been shown to colocalize with endocytosed transferrin, suggesting a similar recycling pathway as in Tf transport (Parker & Parrish, 2000, Suikkanen *et al*., 2002). CPV is released into the cytosol through a mechanism that has been suggested to be transient or to include limited pore formation of the endosomal membrane (Harbison *et al*., 2008). In EV1-infected cells, partial colocalization between EV1 and Rab11, a marker for RE, was seen from 5 min to 30 min p.i. This result suggested that EV1 is transported in TfR-α2I-transfected NIH3T3 cells from EE to RE in a process resembling that of iron transport and also partly in a manner similar to the early infection steps in CPV endocytosis, although CPV remains bound to TfR for several hours (Parker *et al*., 2001).

Other markers used for endocytotic cellular organelles (caveolae/caveosomes and LE/lysosomes) showed no colocalization between either α2I or EV1 in infected cells transfected with a TfR-α2I construct. The markers included caveolin-1, the endoplasmic reticulum marker (PDI) and a Golgi marker (Gm130). For some markers, BHK-21 cells were used because a better fluorescence signal was obtained. CI-MPR, a LE marker, Lamp1, a late endosome and lysosome marker, detected by 1D4B antibody, and and another LE marker 6C4 (30 min to 1 h p.i.) showed no colocalization with α2I or EV1.

These results suggested that EV1 probably remains in the early and recycling endosomes and is not transported further to the late endosomes, lysosomes, Golgi or endoplasmic reticulum.

5.3.4 EV1 replication is initiated in TfR-α2I-transfected NIH3T3 cells

After showing that EV1 was able to enter transiently transfected NIH3T3-TfR-α2I cells, the next focus of interest was to investigate if the virus would be able to initiate productive infection in those cells. In EV1-infected host cells the viral genomic (+)RNA is copied by viral 3D into complementary (-)RNAs, which serve as template for the synthesis of new genomes. RNA replication was measured in infected, TfR-α2I-transfected NIH3T3 cells and Saos-α2 cells by quantitative RT-PCR detecting positive (III, Fig 6 A) or negative (III, Fig. 6 B) strand of viral RNA. In Saos-α2 a considerable increase in (-)RNA copy number was detected (III, Fig. 6 A), as well as a minor increase of (+)RNA (III, Fig. 7 B). This was in line with former studies, where the EV1 RNA synthesis was detected 3 h p.i. and production of new virus started 4h p.i. in the cells (e.g. CV-1 cell line), susceptible to EV1

infection (Pietiäinen *et al*., 2004). In the TfR-α2I-transfected NIH3T3 cells, the increase of both viral (+)RNA and (-)RNA was seen later, first 6 h p.i., thus indicating a slower initiation of replication compared with cells possessing the human α2β1 integrin. The increase of copy number of both (+)RNA and (-)RNA in Saos-α2 cells 12 h p.i, compared with 2 h p.i., was significantly higher compared with TfR-α2I-transfected NIH3T3 cells. Furthermore, in plaque assay experiments, infected TfR-α2I-transfected NIH3T3 cells were shown to produce infectious virus, although in low quantity. Enterovirus replication starts upon release of viral genomic RNA into the cytoplasm, and since the EV1 infection in TfR-α2I-transfected NIH3T3 cells caused production of new viruses, the virus was released from the vesicular structures in the cell. A low pH step is crucial for replication of CPV. In CV-1 cells, EV1 is found in caveosomes 30 min p.i., and viral capsid proteins and RNA can be detected in these structures for 4 h p.i. (Pietiäinen *et al*., 2004). Moreover, in a recent study with Saos-α2β1 cells, caveolin-1 negative tubulovesicular endosomes, containing EV1, were found to fuse with internalized caveolae or caveosomal structure maturating into multivesicular structures, a process that started 15 min p.i and continued for 3 h (Karjalainen *et al*., 2008). Genome release of picornaviruses from intracellular vesicles includes rupture of endosomes by the major rhinovirus receptor group (Schober *et al*., 1998) and pore formation in endosomes by the minor rhinovirus receptor group (Brabec *et al*., 2005). Release of the EV1 genome in transfected NIH3T3-TfR-α2I cells could proceed by rupture of vesicles containing viruses, or alternatively more specific mechanisms could be involved.

Some chimeric receptors have earlier been used in picornavirus studies. For instance, a chimera made of PVR and ICAM-1 or CD4 receptor (Selinka *et al*., 1991) has been shown to be a functional poliovirus receptor (Selinka *et al*., 1992), and expression of a single-chain antibody, recognizing FMDV and inserted to ICAM-1, made cells susceptible to infection (Rieder *et al*., 1996). Together these results show that EV1 initiates productive infection in TfR-α2I-transfected NIH3T3 cells, although the efficiency is reduced.

5.4 HOST GENE EXPRESSION DURING EV1 INFECTION

Previous studies have shown that certain immediate early genes (IEGs) are activated in response to 5-10 h of EV1 infection. The aim was to use cDNA array technique to clarify how the EV1 and α2β1 integrin interaction, signaling and virus endocytosis influence on cellular host gene expression. Host gene RNA expression was studied in human A549 lung carcinoma cells at 30 min, 2 h and 6 h post infection (p.i.).

Picornaviruses may regulate the cells' transcriptional machinery to gain advantage over the host cell in the competition for translational components and ribosomes. At a late stage of enterovirus replication, the infection can be detected by CPE that is characterized

by morphology changes in cell, such as cell rounding and detachment and finally cell lyses (Racaniello, 2001). Picornaviruses lack a 5´-cap structure in their mRNA (Belsham & Sonenberg, 1996, Sachs *et al*., 1997) needed for normal cellular gene expression. Some picornaviruses can inhibit cellular transcription and protein synthesis (shut-off) by preventing the translation of 5´-capped mRNAs (Knipe *et al*., 2001, Lamphear & Rhoads, 1996). The translation of viral genes is not affected due to the internal ribosome entry site (IRES) in the picornavirus genome (Dorner *et al*., 1984, Pelletier & Sonenberg, 1989) that can also be found in some cellular mRNAs.

Confluent A549 (ATCC) cells were infected with EV1 (Farouk strain; ATCC) and the multiplication of the virus was studied by plaque titration (Fig. 1). For RNA purification, the cells were collected after 30 min, 2 h, and 6 h incubation in a guanidinium lysis buffer and frozen at -70°C. Total RNA was purified by ultracentrifugation in CsCl gradients, followed by ethanol precipitation. The preparations were then treated with DNase (Sigma). Alternatively, total RNA was purified according to the manufacturer's instructions using RNeasy Mini kit (Qiagen), supplemented with QIAshredder (Qiagen) for homogenization of cells and RNase-FreeDNase Set (Qiagen) to remove DNA completely during RNA purification. Activation and downregulation of cellular genes was studied by using the Affymetrix Human Genome U133A Array (Affymetrix), that contains 22 215 human cDNA probe sets, representing approximately 19 000 genes. The arrays were scanned, and the fluorescence intensities were measured by Microarray Suite 5.0 software (Affymetrix). The data was transferred to DNA-Chip Analyzer software (Affymetrix) for normalization and model-based analysis (Li et al., 2001). For each probe set, a detection p-value was calculated and sets with $p < 0.04$ were considered to be present, indicating that the gene transcript was reliably detected. Each microarray experiment was repeated. Differently expressed genes were identified by Affymetrix dCHIP software (www.dCHIP.org). Alterations exceeding 1.5 were considered significant in genes where either of the values was greater than 40. To analyze the networks of protein-protein interactions, the STRING 8.2 database was used (Jensen at al., 2009).

In EV1-infected A549 cells, no difference in the amount of RNA in EV1-infected cells, compared with mock-infected cells, was observed at 6 h p.i., although a clear CPE effect in the cells was detected (result not shown). However, at 10 h p.i. the RNA content was significantly decreased in the EV1-infected sample, compared with the mock-infected sample. This could be due to the fact that the CPE in cells decrease the number of viable cells, and/or to virally caused shut-off, although former studies have shown that EV1 causes CPE but does not cause shut-off (Zhang & Racaniello, 1997), or the shut-off is inefficient (Pietiäinen *et al*., 2000). During early stages of EV1 infection at 30 min p.i. and 2 h p.i., only a few host gene expressions were altered. At 6h EV1 p.i., the virus replication had already started and one hundred genes were found to be differently expressed. At this time about 50 % the genes showed decreased expression.

In the early phase of EV1 infection (30 min and 2 h p.i.), the virus is first bound to its cellular receptor, the α2β1 integrin, and subsequently found in vesicular structures. It is possible that the events occurring during virus endocytosis do not require specific alteration of host cell gene expression to enhance initiation of infection. This could explain why only a few genes were differently expressed during these early infection events.

Differently expressed genes at 30 min p.i. in EV1 infected cells possessed functional features, such as actin cytoskeleton, transcription, catalytic activity, cell surface receptors, cell communication and others, including genes with unknown function. Six genes were induced and nine were downregulated. Three of the induced genes in infected cells, including phosphatidylinositol-4-phosphate 5-kinase, type 1C (PIP5K1C), integrin α6 subunit (ITGA6) and receptor-associated coactivator 3 (RAC3) were all shown to be involved in the entry processes of virus. However, their role in EV1 infection requires further studies. STRING 8.2 interactions of the genes did not shown connections between the differently expressed genes, except for a strong acetyl-Coenzyme A acetyltransferase 2 (ACAT2)-acetyl-Coenzyme A acyltransferase 1 (ACAA1) association.

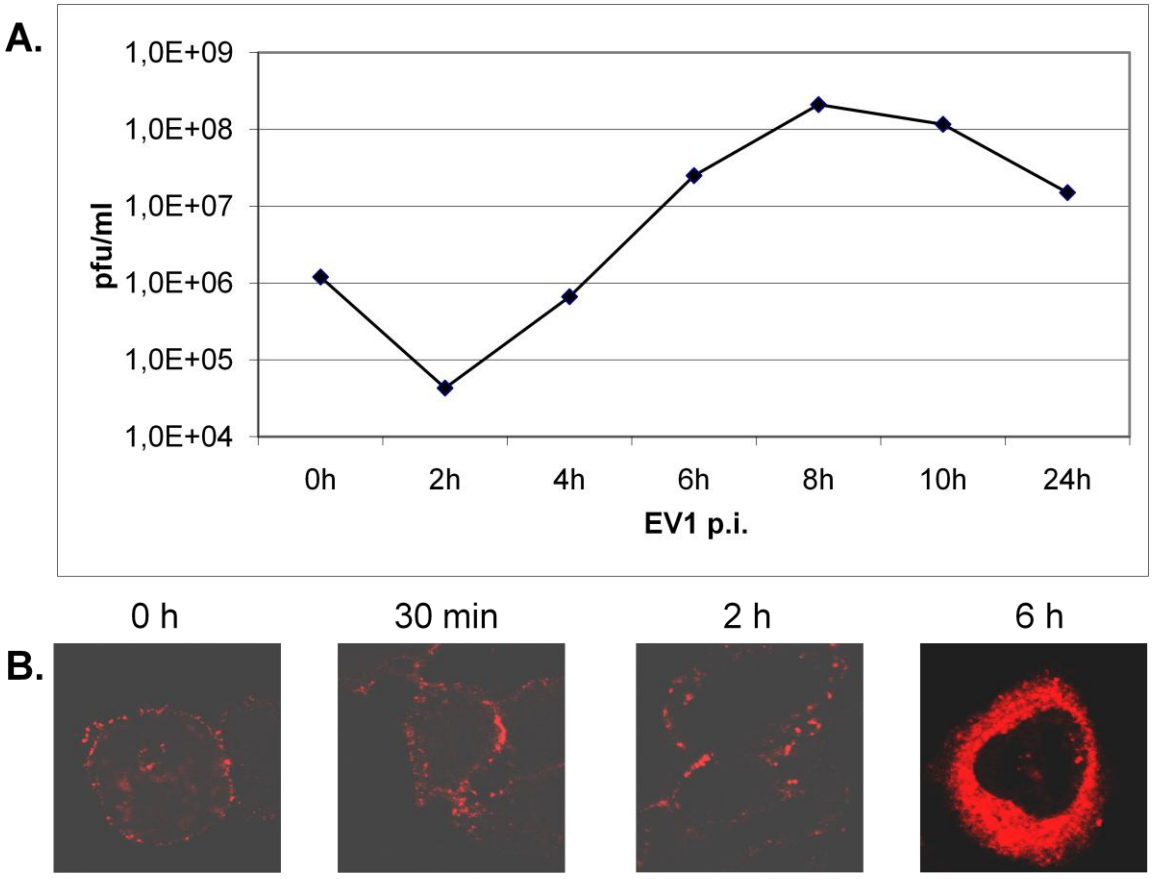


Figure 1. A. The replication cycle of EV1 in A549 cells. The amount of virus was determined by a plaque titration assay. B. IF labelling of EV1 by rabbit antiserum against EV1 in A549 cells at 0 h, 30 min, 2h and 6h p.i.

At 2h EV1 p.i., altered expressed genes included genes involved in cell communication, GTPase activity, JNK cascade, peptidase activity, transcription, anti-apoptosis and others. STRING 8.2 did not show a connection between these genes, but some of these genes, including MAP2K4, CAPN1, IL13RA1 and BCL2A1, could potentially have a role in early infection events and possibly have a role in defense against the virus. However, further investigation into the matter is required.

At 30 min and 2h EV1 p.i., the proteasome subunit beta type 2 (PSMB2) was induced twofold in infected cells, compared to mock-infected cells. PSMB2 is a part of the 20 S proteasome, which is a multicatalytic complex. The 20S proteasome forms a catalytic core in 26S proteasomes. The catalytic core is capped at one or both ends by 19S regulatory particle(s) (Rechsteiner *et al*., 1993, Voges *et al*., 1999). 19S caps structures recognize ubiquitinated proteins, and the substrate is further processed in the catalytic site of the 20S proteasome (Schlax *et al*., 2007). The proteasome is involved in several cellular processes, including stress response (Finley *et al*., 1987) and coordinated protein synthesis regulation, and it generally has an inhibititory effect on translation of cellular mRNA. Proteasome ubiquitination has been shown to be important in the pathogenesis of the enteroviruses CBV3 and PV. CBV3 infection enhances protein ubiquitination, and the ubiquitin-proteasome pathway is needed for optimal replication of the virus (Si *et al*., 2008). The proteasome activity could possibly be involved in host cell stress response to the virus or be part of virus regulation of cellular translation.

In conclusion, the fact that only few genes expression were altered indicate that the virus attachment to receptor and endocytosis cause only minor effects on host cell transcriptional machinery. These results are in line with previous results showing no effect on EV1 expression in HOSpα2AW cells at 1h or 3h p.i. in a human cDNA array containing almost 600 genes (Pietiäinen *et al*., 2000).

At 6h EV1 p.i, the differently expressed genes included several different functional classes, such as genes involved in transcription, regulation of translation, apoptosis, defend respond, cell-cell adhesion, cell cytoskeleton, motility, catalytic activity, cell signaling, IEGs and others, including several genes whose function is not known yet. Analysis of the interactions of the differently expressed genes by STRING 8.2 database (Jensen *et al*., 2009) showed a main cluster of IEGs connected to several other functional genes (Fig 2.). IEGs are a class of genes that show rapid and usually transient activation in the absence of the novo protein synthesis (Platenik *et al*., 2000), in response to a wide variety of cellular stimuli.

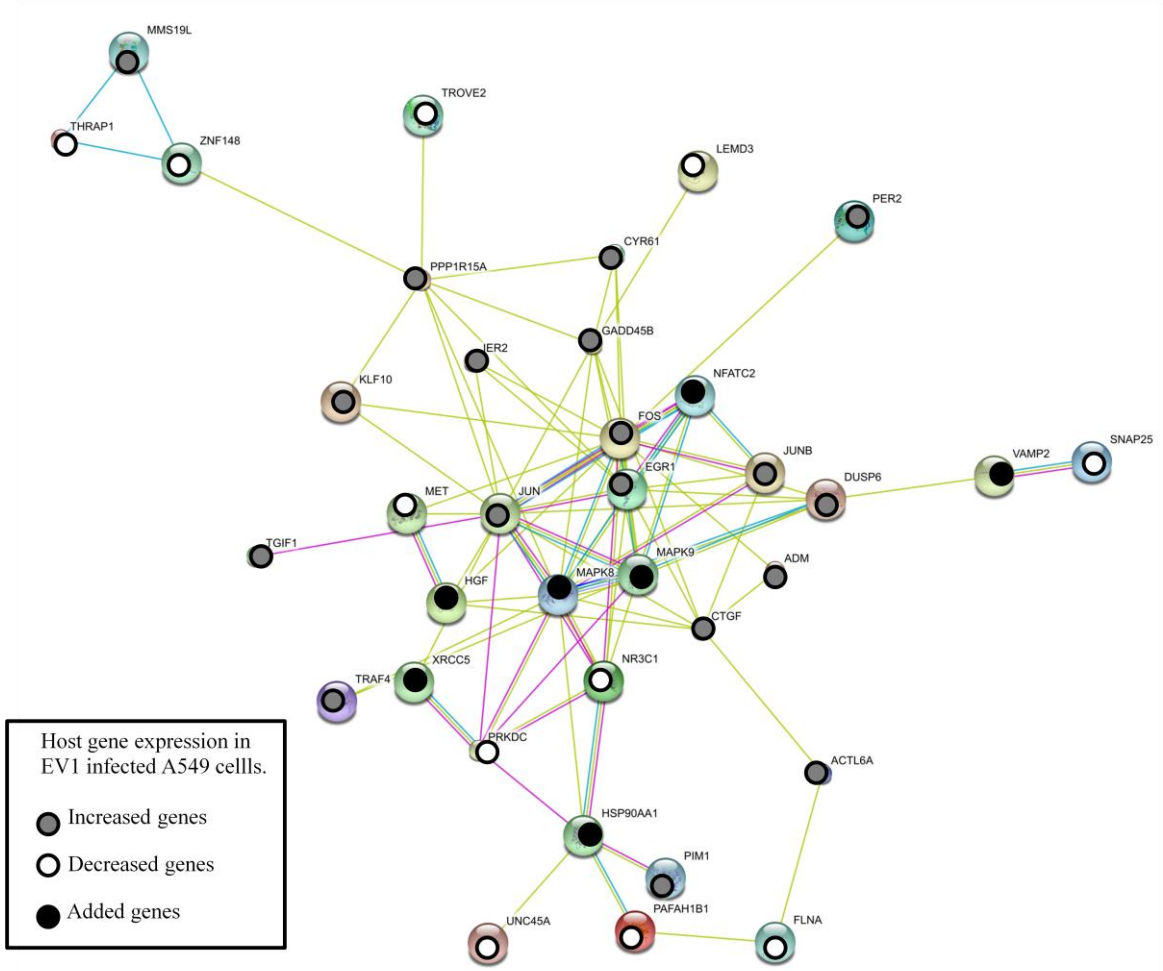


Figure 2. Altered gene products associated with IEGs 6 h after EV1 infection. Induced genes are marked with a grey sphere, decreased genes with a white sphere and associated genes, added based on the STRING 8.2 database, are marked with a black sphere.

No difference in IEG gene expression was observed before 6 h EV1 p.i. Although α2β1 integrin-mediated cell adhesion to type I collagen is known to induce certain IEGs, the activation of IEGs transcription in EV1 infected cells has been shown to require viral replication (Huttunen *et al*., 1997). EV1 6h p.i. in A549 cells induced the expression of several IEGs, including cysteine-rich, angiogenic inducer, 61 (CYR61), JUNB, JUN, immediate early response 2 (IER2), pim-1 oncogene (pim-1), Krueppel-like factor 10 (KLF10), connective tissue growth factor (CTGF) and Polo-like kinase 2 (PLK2) (Table 1). Furthermore, two additional IEGS, early growth response 1 (EGR1) and FOS, were induced during EV1 infection, though they were expressed at low expression values that were slightly under the chosen cut-off level. In former studies many of these IEGs have been reported to be differently expressed during enterovirus infection (Huttunen *et al*., 1997, Huttunen *et al*., 1998, Johannes *et al*., 1999, Kim *et al*., 2004, Lang *et al*., 2008, Pietiäinen *et al*., 2000).

Table 1. Examples of significantly altered gene expression in EV1-infected cells 6 h p.i. The ratios represent values of infected cells (EV1)/uninfected cells (C).

Induced/downregulated gene	Accession No.	EV1	C	Ratio
Murine osteosarcoma viral oncogene homolog (FOS)	NM_005252.2	33	7	+4,8
Early growth response 1 (EGR1)	NM_001964.1	38	11	+3,5
Hairy and enhancer of split 1 (Drosophila) (HES-1)	NM_005524.2	57	19	+3,0
Meningioma expressed antigen 5 (hyaluronidase) (MGEA5)	NM_012215	112	41	+2,7
Protein phosphatase 1, regulatory subunit 15A (PPP1R15A)	NM_014330.2	92	38	+2,4
PIM1 oncogene	NM_002648.1	95	41	+2,3
Actin-like 6A (ACTL6A)	NM_004301.1	55	23	+2,3
Growth arrest and DNA-damage-inducible, beta (GADD45B)	NM_015675.1	82	37	+2,2
Dual specificity phosphatase 6 (DUSP6)	BC003143.1	85	40	+2,1
RNA binding motif protein 5 (RBM5)	NM_005778.1	77	37	+2,1
CDC-like kinase1 (CLK1)	AI251890	45	21	+2,1
Rab geranylgeranyltransferase, beta subunit (RABGGTB)	U49245.1	305	151	+2,0
JUN	NM_002228.2	86	43	+2,0
Vacuolar protein sorting 13 homolog C (S. cerevisiae) (VPS13C)	NM_017684.1	21	57	-2,7
LEM domain containing 3 (LEMD3)	NM_014319.2	18	48	-2,7
Quinoid dihydropteridine reductase (QDPR)	NM_000320.1	57	151	-2,6
Clone RP5-1121G12 on chromosome 20; C20orf104 and SCAND1	AL109965	35	80	-2,2
Chromosome 8 open reading frame 4 (C8ORF4)	NM_020130.1	47	98	-2,1
cDNA DKFZp566I043	AA160522	40	84	-2,1
Chromosome 8 open reading frame 4 (C8ORF4)	NM_020130.1	47	98	-2,0
Cadherin 2, type 1, N-cadherin (neuronal) (CDH2)	NM_001792.1	38	76	-2,0

Cysteine-rich, angiogenic inducer, 61 (CYR61) and connective tissue growth factor (CTGF) both belong to the CCN family of integrin-binding matrix signaling modulators (Moussad & Brigstock, 2000). CYR61 regulates cell proliferation, differentiation, apoptosis, adhesion, migration, apoptosis and extracellular matrix production. Cyr61 expression has been shown to increase during PV infection and CBV3 infection (Johannes *et al*., 1999). Cyr61 could potentially enhance EV1 replication in host cell, since it has been shown to have a positive effect on both CBV3 (Kim *et al*., 2004) and herpes simplex virus 1 (HSV-1) replication (Kurozumi *et al*., 2008), and CYR61 siRNA introduced into Hela cells was able to reduce the CBV3 infection titer (Kim *et al*., 2004). Furthermore, CYR61 is suggested to trigger apoptosis in CBV3-infected Hela cells (Kim *et al*., 2004). CTGF promotes endothelial cell growth, migration, adhesion, and cell survival (Moussad & Brigstock, 2000). In a murine model of chronic CBV3 myocarditis, CTGF was found to be strongly induced (Lang *et al*., 2008). Moreover, in a signaling cascade that has been implicated to be part of diabetic neuropathy, CTGF enhanced the TGFβ/Smad signaling

pathway by transcriptional suppression of Smad7, an antagonist for TGFβ. This was followed by an induction of the transcriptional factor Krueppel-like factor 10 (KLF10) (Wahab *et al*., 2005), an IEG found to be induced in this study. KLF10 was reported to be crucial for the CTGF-mediated downregulation of Smad 7 (Wahab *et al*., 2005). Overexpression of KLF10 has been shown to induce p53 mitochondrial apoptosis, and the level of suppression of this gene is associated with the invasive pattern of breast cancer (Subramaniam *et al*., 1998). Moreover, the expression of KLF10 has been reported to be induced during CBV3 infection in Hela cells (Kim *et al*., 2004) and in EV1 infection 10h p.i. in HOS pα2AW cells (Pietiäinen *et al*., 2000).

Furthermore, CTGF has also been described to be activated by JUN/AP-1 (Yu *et al*., 2009). JUN and JUNB genes are components of the dimeric AP-1 transcription factor that regulates a wide range of cellular processes, such as cell proliferation, death survival and differentiation (Shaulian & Karin, 2002). The transcription of both JUN and JUNB, increased during EV1 replication in this study, and has previously been shown to increase during EV1 and EV7 infection 5-10 h p.i. (Huttunen *et al*., 1997, Huttunen *et al*., 1998, Pietiäinen *et al*., 2000). The EV1 activated stress-related p38 mitogen activated protein kinase (MAPK) and increased the expression of JUNB (Huttunen *et al*., 1998). All or some of the IEGs CYR61, CTGF, KLF10, JUN and JUNB could possibly be involved in the signaling pathway induced by EV1 infection as described above. Some of these genes could also be involved in virus regulation of host genome replication and/or cell survival.

PV infections have been shown to induce both Pim-1 (Johannes et al., 1999, Pietiäinen et al., 2000) and Polo-like kinase 2 (PLK2) (Johannes et al., 1999) expression in host cell. Both PLK2 and Pim-1 could facilitate cell survival in EV1 infection, since they have been shown to activate antiapoptotic pathways in cells (Matsumoto et al., 2009), although PLK2 can also function as a tumor suppressor inducing apotosis (Syed et al., 2006).

The only IEG induced by EV1 infection in this study that to our knowledge has not previously been reported to be differently expressed during enterovirus infection was the immediate early response 2 (IER2) gene. IER2 is suggested to act as a putative transcriptional factor. A possible link to IER2 cell death-inducing potential represents the gene of protein phosphatase 1 and the regulatory (inhibitor) subunit 15A (PPP1R15A) (Kruger *et al*., 2006), the expression of which induced over 2-fold in EV1-infected cells. PPP1R15A is a DNA damage-response gene able to induce apoptosis (Grishin *et al*., 2001). ETR2 has also been found to increase the expression of early growth response 1 (EGR1) in rat fibroblast (Shin *et al*., 2002). EGR1 induced by genotoxic stress was reported to stimulate the expression of growth arrest and DNA-damage-inducible, alpha (GADD45A) and GADD45B and mediate epidermal cell death (Thyss *et al*., 2005).

Table 2. IEG-associated genes listed by function altered during EV1-infetion used in the analysis by the STRING 8.2- database (see Fig.2).

Induced/downregulated gene	Accession No.	EV1	C	Ratio
Transcription				
Murine osteosarcoma viral oncogene homolog (FOS)	NM_005252.2	33	7	+4,8
Early growth response 1 (EGR1)	NM_001964.1	38	11	+3,5
JUN	NM_002228.2	86	43	+2,0
Immediate early response 2 (IER2)	NM_004907.1	566	299	+1,9
Period homolog 2 (Drosophila) (PER2)	NM_022817.1	86	48	+1,8
Homeobox protein TGIF1 (5'-TG-3'-interacting factor 1) (TGIF1)	NM_003244.1	314	194	+1,6
jun B proto-oncogene (JUNB)	NM_002229.1	103	62	+1,6
MMS19-like (MET18 homolog, S. cerevisiae) (MMS19L)	NM_022362.1	76	46	+1,6
Krueppel-like factor 10 (KLF10)	NM_005655.1	201	134	+1,5
Zinc finger protein 148 (ZNF148)	NM_021964.1	28	47	-1,6
TROVE domain family, member 2 (TROVE2)	M25077.1	129	217	-1,7
nuclear receptor subfamily 3, group C, member 1 (NR3C1)	X03348.1	115	188	-1,6
THRAP1 - Thyroid hormone receptor-associated protein complex	NM_005121.1	66	102	-1,5
Apoptosis				
Protein phosphatase 1, regulatory (inhibitor) subunit 15A (PPP1R15A)	NM_014330.2	92	38	+2,4
TNF receptor-associated factor 4 (TRAF4)	NM_004295.1	65	39	+1,7
Growth arrest and DNA-damage-inducible, beta (GADD45B)	NM_015675.1	82	37	+2,2
Dual specificity phosphatase 6 (DUSP6)	BC003143.1	85	40	+2,1
Defence respond				
FOS - FBJ murine osteosarcoma viral oncogene homolog	NM_005252.2	33	7	+4,8
Nuclear receptor subfamily 3, group C, member 1 (NR3C1)	X03348.1	115	187	-1,6
Zinc finger protein 148 (ZNF148)	NM_021964.1	28	47	-1,6
Catalytic activity				
Protein kinase activity				
Pim-1 oncogene	NM_002648.1	95	41	+2,3
Polo-like kinase 2 (Drosophila) (PLK2)	NM_006622.1	209	116	+1,8
MMS19-like (MET18 homolog, S. cerevisiae) (MMS19L)	NM_022362.1	76	46	+1,6
Met proto-oncogene (MET) (hepatocyte growth factor receptor)	U19348.1	77	132	-1,7
Protein kinase, DNA-activated, catalytic polypeptide(PRKDC)	NM_006904.5	115	184	-1,6
Other genes possessing catalytic activity				
Dual specificity phosphatase 6 (DUSP6)	BC003143.1	85	40	+2,1
Platelet-activating factor acetylhydrolase, isoform Ib, subunit 1 (45kDa) (PAFAH1B1)	NM_000430	71	129	-1,8
cytoskeleton/motility				
Actin-like 6A (ACTL6A)	NM_004301.1	55	23	+2,3
Connective tissue growth factor (CTGF)	M92934.1	66	34	+1,9
Platelet-activating factor acetylhydrolase, isoform Ib, alpha subunit 45kDa (PAFAH1B1)	NM_000430	71	130	-1,8
Filamin A, alpha (actin-binding protein-280) (FLNA)	NM_001456	139	252	-1,8
IEGs				
FOS - FBJ murine osteosarcoma viral oncogene homolog	NM_005252.2	33	7	+4,8
Early growth response 1 (EGR1)	NM_001964.1	38	11	+3,5
Pim-1 oncogene (Pim-1)	NM_002648.1	95	41	+2,3
JUN	NM_002228.2	86	43	+2,0
Immediate early response 2 (IER2)	NM_004907.1	566	299	+1,9

Connective tissue growth factor (CTGF)	M92934.1	66	34	+1,9
Polo-like kinase 2 (PLK2)	NM_006622.1	209	116	+1,8
Cysteine-rich, angiogenic inducer, 61 (CYR61)	NM_001554.1	125	79	+1,6
jun B proto-oncogene (JUNB)	NM_002229.1	103	62	+1,6
Krueppel-like factor 10 (KLF10)	NM_005655.1	201	134	+1,5
Other				
Adrenomedullin (ADM)	NM_001124.1	583	346	+1,7
Ribosomal RNA processing 7 homolog A (S. cerevisiae) (RRP7A)	NM_015703.1	128	82	+1,6
LEM domain containing 3 (LEMD3)	NM_014319.2	18	48	-2,7
Synaptosomal-associated protein, 25kD (SNAP25)	NM_003081.1	60	109	-1,8

GADD45B was induced by EV1-infected A549 cells, and both GADD45A and GADD45B genes activate cell death in cells failing in DNA repair. In a former study, EGR1 expression increased at 6 h p.i. in EV1-infected HOS pα2AW, and the induction was increased over two-fold at 10h p.i. Furthermore, at this time point the GADD45A gene was also induced (Pietiäinen *et al*., 2000). This represents two possible pathways by which EV1 could regulate cell survival by activating the ETR2 gene. In addition, further examples of mechanisms by which EV1 could possibly control cell survival included cell regulation mechanisms of some altered genes reported to either suppress or induce apoptosis. In EV1-infected cells the expression of the genes RNA binding motif protein 5 (RBM5) and dual specificity phosphatase 6 (DUSP6), of which both have been reported to activate apoptosis, were over 2-fold increased (Table 2). In a former study, upregulation of cellular expression of RBM5 in A549 cells was shown to suppress cell proliferation by increasing apoptosis and inducing the cell cycle arrest in G_1 (Oh *et al*., 2006). Together these implicated some possible mechanisms by which EV1 could regulate the host cell survival of A549 cells. However, this needs to be analyzed in further studies. A study by Huttunen et al. suggested that a minor part of the HOS pα_2AW cells undergo apoptosis during infection by EV1 at 12 h p.i. or by Semliki Forrest virus at 24 h p.i. (Huttunen *et al*., 1998), a virus shown to induce apoptosis in several cell lines (Glasgow *et al*., 1997, Scallan *et al*., 1997).

Our study also suggested possible roles for genes involved in cytoskeleton and motility during EV1 infection in A549 cells. This involved platelet-activating factor acetylhydrolase, isoform Ib, alpha subunit 45kDa (PAFAH1B1), and filamin A (FLNA) linked to IEGs by predicted interaction pathway by STRING 8.2 database (Jensen *et al*., 2009) (Fig. 2).

In a study by Kondratova et al, PV 3A protein was described to inhibit the action of PAFAH1B1 by blocking PAFAH1B1-containing complexes on the cytoplasmic surface of the ER and the Golgi, thereby blocking the vesicular transport from ER to Golgi. This event leads to prompt disappearance of short-living receptors from plasma membrane and loss of cell sensitivity to TNF and interferon (Kondratova *et al*., 2005). A possible role of

PAFAH1B1 in neuronal disease caused by PV infection has also been suggested, since neurons are most sensitive to defiance of PAFAH1B1 protein activity and decrease of PAFAH1B1 concentration (Fogli *et al*., 1999). PAFAH1B1 may also protect EV1 from host defence by a mechanism similar to the one reported for PV, and it could be speculated whether this gene could also play a role in EV1 neuronal infections.

In conclusion, several IEGs were expressed in a similar pattern in EV1 infected A549 cells as reported in earlier studies for EV1 and also for other enteroviruses (Huttunen *et al*., 1997, Huttunen *et al*., 1998, Johannes *et al*., 1999, Kim *et al*., 2004, Lang *et al*., 2008, Pietiäinen *et al*., 2000). Together these results suggest a central role for certain IEGs in signaling pathways induced by EV1 replication. During the EV1 replication pattern several differently expressed genes, known to play a role in apoptosis, seemed to favor apoptosis over cell survival, although some specific gene expressions also suggested the possibility of antiapoptotic events in the cell. Furthermore, differently expressed genes involved in cytoskeleton and motility could possibly affect the host cell by negatively affecting the structural integrity of the cell, and thus lead to growth arrest and hamper host cell defense. However, further studies are needed to clarify the role of these genes in EV1 replication.

6 CONCLUSIONS

Integrins are a family of cell adhesion receptors that mainly recognize ECM ligands and cell-surface ligands, but some soluble ligands have also been reported. Integrins have been implicated in various diseases and disease processes, including inflammation, atherosclerosis and cancer. Moreover, several viral pathogens, including enteroviruses, use integrins as cellular receptors. Enteroviruses have also been suggested to be involved in the etiopathogenesis of type 1 diabetes. The thesis focused on studying the role of integrins in the pathogenesis of metastasis to cortical bone, type 1 diabetes and echovirus 1 infection.

Favored adherence of breast cancer cells to bone-specific factors may facilitate preferential metastasis to the skeleton. The most important integrins in the initial attachment of breast cancer cells to bone were studied in an in vitro microplate attachment assay, measuring the binding of MDA-MD-231 breast cancer cells to bovine cortical bone disks. The results suggest α2β1 and α3β1 to be the key receptors in the initial anchoring of breast cancer cells to cortical bone matrix.

Enterovirus infection has been suggested to be associated with the pathogenesis of T1D. Increased prevalence and higher enterovirus antibody levels, as well as presence of enterovirus RNA in blood, have been reported in individuals with recent onset T1D. The possibility that sensitivity to enterovirus infections could correlate with individual variation in enterovirus receptor genes, including some integrins, was investigated by sequence analysis. The predicted polypeptide domains known to be critical for virus binding in the major enterovirus receptors were determined. In the PVR, an SNP causing an amino acid change was detected in four individuals with T1D. The patients also had a lower level of neutralizing antibody against echovirus 30, compared to healthy children. However, the findings of direct connection to T1D should be analyzed in a larger cohort to obtain statistically sustainable results.

Echovirus 1 (EV1) binds to the α2 I domain (α2I) of the human α2β1 integrin. EV1 has been reported to enter a host cell either via lipid rafts, by macropinocytosis or, through caveolae. To determine if EV1 could initiate productive infection by using chlathrin-directed entry, a chimeric receptor was constructed by inserting α2I into the extracellular terminus of transferrin receptor (TfR), responsible for transport of transferrin-bound iron through clathrin-dependent endocytosis. In immunofluorescence and confocal microscopy studies, the TfR-α2I chimeric receptor when transfected into non-permissive cells, was localized to the cell surface and also in vesicular structures in the cytoplasm. EV1 was able to bind the chimeric receptor and, consequently, the chimeric virus receptor complex was internalized through the clathrin-mediated endocytosis to the early endosomes and recycling endosomes. After 1 h, the chimeric receptor was completely separated from the

virus as it was recycled to the plasma membrane, whereas the virus remained in intracellular vesicles. Based on quantitative RT-PCR detecting both positive and negative strand viral RNA, the replication cycle of EV1 was initiated in the cells carrying the chimeric receptor. Furthermore, in plaque assay experiments infected TfR-α2I-transfected NIH3T3 cells were shown to produce infectious virus, although in low quantities. The results indicate that the natural entry route is not an absolute requirement for EV1 replication; instead, the virus can adapt to use alternative internalization mechanisms.

Previous studies have shown that certain immediate early genes (IEGs) are activated in response to EV1 infection. To elucidate the effect of EV1 and α2β1 integrin interaction, virus endocytosis and replication in human A549 lung carcinoma cells, cDNA microarray analysis was performed. In samples collected during virus endocytosis (30 min and 2h p.i.) only a few genes were differently expressed. Six hours after initiation of the infection, a cytopathic effect was observed in the cells and approximately 100 genes (0.53%) of those investigated were differently expressed, including IEGs, genes affecting the host cell immune response and apoptosis. Several of the induced IEGs, such as CYR61, JUNB, JUN, PIM1, KLF10, CTGF and PLK2, have been reported to be expressed during infection of other enteroviruses. STRING-analysis of this gene pattern detected cluster, consisting mainly of IEGs, suggested IEGs to be primarily associated with EV1 replication in host cell.

ACKNOWLEDGEMENTS

This study was carried out at the Department of Virology, University of Turku; Department of Virology, Haartman Institute, University of Helsinki; and in the Enterovirus Laboratory, National Public Health Institute, Helsinki. I would like to thank Professor Emeritus Antti Vaheri, who was the Head of the Department of Virology at the beginning of the study, as well as the present Head of the Department, Professor Kalle Saksela, for the excellent facilities the institute has provided. I also wish to thank Jussi Huttunen, Head of the National Public Health Institute, for providing excellent facilities.

I also want to thank my thesis supervisor, Professor Timo Hyypiä, for the opportunity to carry out this study as a member of his excellent research team and for introducing me to the world of picornaviruses. I also thank him for his skilful and generous guidance during the writing process of the thesis.

In addition, I wish to thank the reviewers of my thesis, Professor Klaus Hedman and Docent Arno Hänninen for their hard work and valuable comments on the manuscript that greatly helped to improve it. I also want to thank proof-reader Outi Huovinen for revising the language of this thesis. I also thank Professor Klaus Hedman and Dr Janna Saarela for giving valuable comments and ideas as members of my thesis committee.

I particularly wish to thank the supervisor of my master's thesis and co-author of my first papers, Tommy Nordström, for his excellent guidance, positive attitude and stimulating discussion that helped to clarify the background of the research.

I also want to thank our collaborator, Docent Merja Roivainen, for giving me the opportunity to carry out the study in her laboratory, her positive attitude and for always having time to give advice.

In addition, I wish to thank all my other co-authors, Docent Varpu Marjomäki, Professor Jorma Ilonen, Professor Christer Lindqvist, Professor Outi Vaarala, Professor Tapani Hovi, Docent Matti Waris, Dr Johanna Holmbom and Professor Jyrki Heino for their valuable contribution.

I also wish to thank all the former and present members of the picornavirus research group, including Anne, Annu, Camilla, Hannamari, Heidi, Heli, Juhana, Leena, Maaria, Maarit, Olya, Outi, Pasi, Petri, Pia, Päivi, Seija, Tiiu, Tommi, Tuija, Veera, Vilja and many others. Thank you for creating such a wonderful working atmosphere and organizing memorable events outside the laboratory. Special thanks to Tuija for patiently answering all my questions and for her valuable help with laboratory work.

I also want to acknowledge Mervi Eskelinen and Leena Kostamovara for their generous help with practical laboratory problems.

Special thanks to my mother-in-law Leena Karttunen for being such a good grandmother to my children. She really helped me finish my thesis by spending time with the children.

My parents: Thank you for always being there for me. My mother: I wish you could still be here today and I miss you. My sisters and brothers: Thank you for your friendship

and support. Thanks to all my friends outside the laboratory for your support and inspiring discussions.

My warmest thanks go to Jussi, Kasper and Selma. Thank you, Jussi for your endless support and love. Kasper and Selma, thank you for giving me happiness and joy.

This thesis was financially supported by grants from Paulo Foundation, the Sigrid Juselius Foundation, Suomen Kulttuurirahasto, the Helsinki Biomedical Graduate School, Yrjö Jahnsson Foundation, Farmos Research and Science Foundation.

REFERENCES

[Anonymous]. (2000). Infections and vaccinations as risk factors for childhood type I (insulin-dependent) diabetes mellitus: A multicentre case-control investigation. EURODIAB substudy 2 study group. *Diabetologia* **43**, 47-53.

[Anonymous]. (1955). ENTERIC cytopathogenic human orphan (ECHO) viruses. *Science* **122**, 1187-1188.

Abiru N., Kawasaki E., Eguch K. (2002). Current knowledge of japanese type 1 diabetic syndrome. *Diabetes Metab Res Rev* **18**, 357-366.

Abraham G. & Colonno R. J. (1984). Many rhinovirus serotypes share the same cellular receptor. *J Virol* **51**, 340-345.

Akalu A., Cretu A., Brooks P. C. (2005). Targeting integrins for the control of tumour angiogenesis. *Expert Opin Investig Drugs* **14**, 1475-1486.

Akerblom H. K., Vaarala O., Hyöty H., Ilonen J., Knip M. (2002). Environmental factors in the etiology of type 1 diabetes. *Am J Med Genet* **115**, 18-29.

Albini A., Benelli R., Noonan D. M., Brigati C. (2004). The "chemoinvasion assay": A tool to study tumor and endothelial cell invasion of basement membranes. *Int J Dev Biol* **48**, 563-571.

Alexander D. A. & Dimock K. (2002). Sialic acid functions in enterovirus 70 binding and infection. *J Virol* **76**, 11265-11272.

Alford D. & Taylor-Papadimitriou J. (1996). Cell adhesion molecules in the normal and cancerous mammary gland. *J Mammary Gland Biol Neoplasia* **1**, 207-218.

Alford D., Pitha-Rowe P., Taylor-Papadimitriou J. (1998). Adhesion molecules in breast cancer: Role of alpha 2 beta 1 integrin. *Biochem Soc Symp* **63**, 245-259.

Amstutz B., Gastaldelli M., Kalin S., Imelli N., Boucke K., Wandeler E., Mercer J., Hemmi S., Greber U. F. (2008). Subversion of CtBP1-controlled macropinocytosis by human adenovirus serotype 3. *EMBO J* **27**, 956-969.

Amyere M., Payrastre B., Krause U., Van Der Smissen P., Veithen A., Courtoy P. J. (2000). Constitutive macropinocytosis in oncogene-transformed fibroblasts depends on sequential permanent activation of phosphoinositide 3-kinase and phospholipase C. *Mol Biol Cell* **11**, 3453-3467.

Anders M., Christian C., McMahon M., McCormick F., Korn W. M. (2003). Inhibition of the Raf/MEK/ERK pathway up-regulates expression of the coxsackievirus and adenovirus receptor in cancer cells. *Cancer Res* **63**, 2088-2095.

Anderson H. A., Chen Y., Norkin L. C. (1996). Bound simian virus 40 translocates to caveolin-enriched membrane domains, and its entry is inhibited by drugs that selectively disrupt caveolae. *Mol Biol Cell* **7**, 1825-1834.

Anderson R. G. (1998). The caveolae membrane system. *Annu Rev Biochem* **67**, 199-225.

Andreoletti L., Hober D., Hober-Vandenberghe C., Belaich S., Vantyghem M. C., Lefebvre J., Wattre P. (1997). Detection of coxsackie B virus RNA sequences in whole blood samples from adult patients at the onset of type I diabetes mellitus. *J Med Virol* **52**, 121-127.

Angata T. & Varki A. (2002). Chemical diversity in the sialic acids and related alpha-keto acids: An evolutionary perspective. *Chem Rev* **102**, 439-469.

Aquilina A., Korda M., Bergelson J. M., Humphries M. J., Farndale R. W., Tuckwell D. (2002). A novel gain-of-function mutation of the integrin alpha2 VWFA domain. *Eur J Biochem* **269**, 1136-1144.

Arends M. J., Morris R. G., Wyllie A. H. (1990). Apoptosis. the role of the endonuclease. *Am J Pathol* **136**, 593-608.

Arihiro K., Inai K., Kurihara K., Takeda S., Kaneko M., Kuroi K., Toge T. (1993). Loss of VLA-2 collagen receptor in breast carcinoma, facilitating invasion and metastasis. *Jpn J Cancer Res* **84**, 726-733.

Arnaout M. A., Mahalingam B., Xiong J. P. (2005). Integrin structure, allostery, and bidirectional signaling. *Annu Rev Cell Dev Biol* **21**, 381-410.

Atkinson M. A. & Leiter E. H. (1999). The NOD mouse model of type 1 diabetes: As good as it gets? *Nat Med* **5**, 601-604.

Atkinson M. A., Bowman M. A., Campbell L., Darrow B. L., Kaufman D. L., Maclaren N. K. (1994). Cellular immunity to a determinant common to glutamate decarboxylase and coxsackie virus in insulin-dependent diabetes. *J Clin Invest* **94**, 2125-2129.**Au G. G., Lincz L. F., Enno A., Shafren D. R. (2007).** Oncolytic coxsackievirus A21 as a novel therapy for multiple myeloma. *Br J Haematol* **137**, 133-141.

Autret A., Martin-Latil S., Brisac C., Mousson L., Colbere-Garapin F., Blondel B. (2008). Early phosphatidylinositol 3-kinase/Akt pathway activation limits poliovirus-induced JNK-mediated cell death. *J Virol* **82**, 3796-3802.

Autret A., Martin-Latil S., Mousson L., Wirotius A., Petit F., Arnoult D., Colbere-Garapin F., Estaquier J., Blondel B. (2007). Poliovirus induces bax-dependent cell death mediated by c-jun NH2-terminal kinase. *J Virol* **81**, 7504-7516.

Babbey C. M., Ahktar N., Wang E., Chen C. C., Grant B. D., Dunn K. W. (2006). Rab10 regulates membrane transport through early endosomes of polarized madin-darby canine kidney cells. *Mol Biol Cell* **17**, 3156-3175.

Babuke T. & Tikkanen R. (2007). Dissecting the molecular function of reggie/flotillin proteins. *Eur J Cell Biol* **86**, 525-532.

Baekkeskov S., Aanstoot H. J., Christgau S., Reetz A., Solimena M., Cascalho M., Folli F., Richter-Olesen H., De Camilli P. (1990). Identification of the 64K autoantigen in insulin-dependent diabetes as the GABA-synthesizing enzyme glutamic acid decarboxylase. *Nature* **347**, 151-156.

Banno A. & Ginsberg M. H. (2008). Integrin activation. *Biochem Soc Trans* **36**, 229-234.

Baranowski E., Ruiz-Jarabo C. M., Sevilla N., Andreu D., Beck E., Domingo E. (2000). Cell recognition by foot-and-mouth disease virus that lacks the RGD integrin-binding motif: Flexibility in aphthovirus receptor usage. *J Virol* **74**, 1641-1647.

Barberis L., Wary K. K., Fiucci G., Liu F., Hirsch E., Brancaccio M., Altruda F., Tarone G., Giancotti F. G. (2000). Distinct roles of the adaptor protein shc and focal adhesion kinase in integrin signaling to ERK. *J Biol Chem* **275**, 36532-36540.

Barrett-Connor E. (1985). Is insulin-dependent diabetes mellitus caused by coxsackievirus B infection? A review of the epidemiologic evidence. *Rev Infect Dis* **7**, 207-215.

Bar-Sagi D. & Feramisco J. R. (1986). Induction of membrane ruffling and fluid-phase pinocytosis in quiescent fibroblasts by ras proteins. *Science* **233**, 1061-1068.

Bar-Sagi D., McCormick F., Milley R. J., Feramisco J. R. (1987). Inhibition of cell surface ruffling and fluid-phase pinocytosis by microinjection of anti-ras antibodies into living cells. *J Cell Physiol Suppl* **Suppl 5**, 69-73.

Baum O., Hlushchuk R., Forster A., Greiner R., Clezardin P., Zhao Y., Djonov V., Gruber G. (2007). Increased invasive potential and up-regulation of MMP-2 in MDA-MB-231 breast cancer cells expressing the beta3 integrin subunit. *Int J Oncol* **30**, 325-332.

Belsham G. J. & Sonenberg N. (1996). RNA-protein interactions in regulation of picornavirus RNA translation. *Microbiol Rev* **60**, 499-511.

Benmerah A., Bayrou M., Cerf-Bensussan N., Dautry-Varsat A. (1999). Inhibition of clathrin-coated pit assembly by an Eps15 mutant. *J Cell Sci* **112 (Pt 9)**, 1303-1311.

Berg A. K., Korsgren O., Frisk G. (2006). Induction of the chemokine interferon-gamma-inducible protein-10 in human pancreatic islets during enterovirus infection. *Diabetologia* **49**, 2697-2703.

Bergelson J. M., Chan B. M., Finberg R. W., Hemler M. E. (1993). The integrin VLA-2 binds echovirus 1 and extracellular matrix ligands by different mechanisms. *J Clin Invest* **92**, 232-239.

Bergelson J. M., Shepley M. P., Chan B. M., Hemler M. E., Finberg R. W. (1992). Identification of the integrin VLA-2 as a receptor for echovirus 1. *Science* **255**, 1718-1720.

Bergelson J. M., Mohanty J. G., Crowell R. L., St John N. F., Lublin D. M., Finberg R. W. (1995). Coxsackievirus B3 adapted to growth in RD cells binds to decay-accelerating factor (CD55). *J Virol* **69**, 1903-1906.

Bergelson J. M., Chan M., Solomon K. R., St John N. F., Lin H., Finberg R. W. (1994). Decay-accelerating factor (CD55), a glycosylphosphatidylinositol-anchored complement regulatory protein, is a receptor for several echoviruses. *Proc Natl Acad Sci U S A* **91**, 6245-6248.

Bergelson J. M., St John N., Kawaguchi S., Chan M., Stubdal H., Modlin J., Finberg R. W. (1993). Infection by echoviruses 1 and 8 depends on the alpha 2 subunit of human VLA-2. *J Virol* **67**, 6847-6852.

Bergelson J. M., Cunningham J. A., Droguett G., Kurt-Jones E. A., Krithivas A., Hong J. S., Horwitz M. S., Crowell R. L., Finberg R. W. (1997). Isolation of a common receptor for coxsackie B viruses and adenoviruses 2 and 5. *Science* **275**, 1320-1323.

Berger M. M., Kopp N., Vital C., Redl B., Aymard M., Lina B. (2000). Detection and cellular localization of enterovirus RNA sequences in spinal cord of patients with ALS. *Neurology* **54**, 20-25.

Berinstein A., Roivainen M., Hovi T., Mason P. W., Baxt B. (1995). Antibodies to the vitronectin receptor (integrin alpha V beta 3) inhibit binding and infection of foot-and-mouth disease virus to cultured cells. *J Virol* **69**, 2664-2666.

Berry L. J., Au G. G., Barry R. D., Shafren D. R. (2008). Potent oncolytic activity of human enteroviruses against human prostate cancer. *Prostate* **68**, 577-587.

Betts P., Mulligan J., Ward P., Smith B., Wilkin T. (2005). Increasing body weight predicts the earlier onset of insulin-dependant diabetes in childhood: Testing the 'accelerator hypothesis' (2). *Diabet Med* **22**, 144-151.

Beyan H., Valorani M. G., Pozzilli P. (2003). --To: Gale EAM (2002) A missing link in the hygiene hypothesis? diabetologia 45:588-592. *Diabetologia* **46**, 301-2; author reply 302.

Bhella D., Goodfellow I. G., Roversi P., Pettigrew D., Chaudhry Y., Evans D. J., Lea S. M. (2004). The structure of echovirus type 12 bound to a two-domain fragment of its cellular attachment protein decay-accelerating factor (CD 55). *J Biol Chem* **279**, 8325-8332.

Birk O. S., Douek D. C., Elias D., Takacs K., Dewchand H., Gur S. L., Walker M. D., van der Zee R., Cohen I. R., Altmann D. M. (1996). A role of Hsp60 in autoimmune diabetes: Analysis in a transgenic model. *Proc Natl Acad Sci U S A* **93**, 1032-1037.

Bjorkman P. J., Saper M. A., Samraoui B., Bennett W. S., Strominger J. L., Wiley D. C. (1987). Structure of the human class I histocompatibility antigen, HLA-A2. *Nature* **329**, 506-512.

Blom L., Nyström L., Dahlquist G. (1991). The swedish childhood diabetes study. vaccinations and infections as risk determinants for diabetes in childhood. *Diabetologia* **34**, 176-181.

Boissier S., Magnetto S., Frappart L., Cuzin B., Ebetino F. H., Delmas P. D., Clezardin P. (1997). Bisphosphonates inhibit prostate and breast carcinoma cell adhesion to unmineralized and mineralized bone extracellular matrices. *Cancer Res* **57**, 3890-3894.

Boles K. S., Vermi W., Facchetti F., Fuchs A., Wilson T. J., Diacovo T. G., Cella M., Colonna M. (2009). A novel molecular interaction for the adhesion of follicular CD4 T cells to follicular DC. *Eur J Immunol* **39**, 695-703.

Boonyakiat Y., Hughes P. J., Ghazi F., Stanway G. (2001). Arginine-glycine-aspartic acid motif is critical for human parechovirus 1 entry. *J Virol* **75**, 10000-10004.

Bottger G., Nagelkerken B., van der Sluijs P. (1996). Rab4 and Rab7 define distinct nonoverlapping endosomal compartments. *J Biol Chem* **271**, 29191-29197.

Bottger T. C., Maschek H., Lobo M., Gottwohl R. G., Brenner W., Junginger T. (1999). Prognostic value of immunohistochemical expression of beta-1 integrin in pancreatic carcinoma. *Oncology* **56**, 308-313.

Bottino C., Castriconi R., Pende D., Rivera P., Nanni M., Carnemolla B., Cantoni C., Grassi J., Marcenaro S.& other authors. (2003). Identification of PVR (CD155) and nectin-2 (CD112) as cell surface ligands for the human DNAM-1 (CD226) activating molecule. *J Exp Med* **198**, 557-567.

Brabec M., Baravalle G., Blaas D., Fuchs R. (2003). Conformational changes, plasma membrane penetration, and infection by human rhinovirus type 2: Role of receptors and low pH. *J Virol* **77**, 5370-5377.

Brabec M., Schober D., Wagner E., Bayer N., Murphy R. F., Blaas D., Fuchs R. (2005). Opening of size-selective pores in endosomes during human rhinovirus serotype 2 in vivo uncoating monitored by single-organelle flow analysis. *J Virol* **79**, 1008-1016.

Brandenburg B., Lee L. Y., Lakadamyali M., Rust M. J., Zhuang X., Hogle J. M. (2007). Imaging poliovirus entry in live cells. *PLoS Biol* **5**, e183.

Brodsky F. M., Chen C. Y., Knuehl C., Towler M. C., Wakeham D. E. (2001). Biological basket weaving: Formation and function of clathrin-coated vesicles. *Annu Rev Cell Dev Biol* **17**, 517-568.

Brooks P. C., Strömblad S., Sanders L. C., von Schalscha T. L., Aimes R. T., Stetler-Stevenson W. G., Quigley J. P., Cheresh D. A. (1996). Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin alpha v beta 3. *Cell* **85**, 683-693.

Brown D. A. & London E. (1998). Functions of lipid rafts in biological membranes. *Annu Rev Cell Dev Biol* **14**, 111-136.

Brown G., Aitken J., Rixon H. W., Sugrue R. J. (2002). Caveolin-1 is incorporated into mature respiratory syncytial virus particles during virus assembly on the surface of virus-infected cells. *J Gen Virol* **83**, 611-621.

Bucci C., Thomsen P., Nicoziani P., McCarthy J., van Deurs B. (2000). Rab7: A key to lysosome biogenesis. *Mol Biol Cell* **11**, 467-480.

Bucci C., Parton R. G., Mather I. H., Stunnenberg H., Simons K., Hoflack B., Zerial M. (1992). The small GTPase rab5 functions as a regulatory factor in the early endocytic pathway. *Cell* **70**, 715-728.

Buenz E. J. & Howe C. L. (2006). Picornaviruses and cell death. *Trends Microbiol* **14**, 28-36.

Cacciari B. & Spalluto G. (2005). Non peptidic alphavbeta3 antagonists: Recent developments. *Curr Med Chem* **12**, 51-70.

Cainelli F., Manzaroli D., Renzini C., Casali F., Concia E., Vento S. (2000). Coxsackie B virus-induced autoimmunity to GAD does not lead to type 1 diabetes. *Diabetes Care* **23**, 1021-1022.

Camper L., Hellman U., Lundgren-Akerlund E. (1998). Isolation, cloning, and sequence analysis of the integrin subunit alpha10, a beta1-associated collagen binding integrin expressed on chondrocytes. *J Biol Chem* **273**, 20383-20389.

Camper L., Heinegård D., Lundgren-Akerlund E. (1997). Integrin alpha2beta1 is a receptor for the cartilage matrix protein chondroadherin. *J Cell Biol* **138**, 1159-1167.

Carter D. R. & Spengler D. M. (1978). Mechanical properties and composition of cortical bone. *Clin Orthop Relat Res* **(135)**, 192-217.

Carthy C. M., Granville D. J., Watson K. A., Anderson D. R., Wilson J. E., Yang D., Hunt D. W., McManus B. M. (1998). Caspase activation and specific cleavage of substrates after coxsackievirus B3-induced cytopathic effect in HeLa cells. *J Virol* **72**, 7669-7675.

Chadda R., Howes M. T., Plowman S. J., Hancock J. F., Parton R. G., Mayor S. (2007). Cholesterol-sensitive Cdc42 activation regulates actin polymerization for endocytosis via the GEEC pathway. *Traffic* **8**, 702-717.

Chan B. M., Matsuura N., Takada Y., Zetter B. R., Hemler M. E. (1991). In vitro and in vivo consequences of VLA-2 expression on rhabdomyosarcoma cells. *Science* **251**, 1600-1602.

Chang K. H., Day C., Walker J., Hyypiä T., Stanway G. (1992). The nucleotide sequences of wild-type coxsackievirus A9 strains imply that an RGD motif in VP1 is functionally significant. *J Gen Virol* **73 (Pt 3)**, 621-626.

Chapman H. A., Wei Y., Simon D. I., Waltz D. A. (1999). Role of urokinase receptor and caveolin in regulation of integrin signaling. *Thromb Haemost* **82**, 291-297.

Chappell T. G., Welch W. J., Schlossman D. M., Palter K. B., Schlesinger M. J., Rothman J. E. (1986). Uncoating ATPase is a member of the 70 kilodalton family of stress proteins. *Cell* **45**, 3-13.

Chazal N. & Gerlier D. (2003). Virus entry, assembly, budding, and membrane rafts. *Microbiol Mol Biol Rev* **67**, 226-37, table of contents.

Chehadeh W., Weill J., Vantyghem M. C., Alm G., Lefebvre J., Wattre P., Hober D. (2000). Increased level of interferon-alpha in blood of patients with insulin-dependent diabetes mellitus: Relationship with coxsackievirus B infection. *J Infect Dis* **181**, 1929-1939.

Chen C. C., Schweinsberg P. J., Vashist S., Mareiniss D. P., Lambie E. J., Grant B. D. (2006a). RAB-10 is required for endocytic recycling in the caenorhabditis elegans intestine. *Mol Biol Cell* **17**, 1286-1297.

Chen H., Slepnev V. I., Di Fiore P. P., De Camilli P. (1999). The interaction of epsin and Eps15 with the clathrin adaptor AP-2 is inhibited by mitotic phosphorylation and enhanced by stimulation-dependent dephosphorylation in nerve terminals. *J Biol Chem* **274**, 3257-3260.

Chen T. C., Lai Y. K., Yu C. K., Juang J. L. (2007). Enterovirus 71 triggering of neuronal apoptosis through activation of abl-Cdk5 signalling. *Cell Microbiol* **9**, 2676-2688.

Chothia C. & Jones E. Y. (1997). The molecular structure of cell adhesion molecules. *Annu Rev Biochem* **66**, 823-862.

Chung J. & Mercurio A. M. (2004). Contributions of the alpha6 integrins to breast carcinoma survival and progression. *Mol Cells* **17**, 203-209.

Chung S. K., Kim J. Y., Kim I. B., Park S. I., Paek K. H., Nam J. H. (2005). Internalization and trafficking mechanisms of coxsackievirus B3 in HeLa cells. *Virology* **333**, 31-40.

Ciarlet M., Crawford S. E., Cheng E., Blutt S. E., Rice D. A., Bergelson J. M., Estes M. K. (2002). VLA-2 (alpha2beta1) integrin promotes rotavirus entry into cells but is not necessary for rotavirus attachment. *J Virol* **76**, 1109-1123.

Clague M. J., Urbe S., Aniento F., Gruenberg J. (1994). Vacuolar ATPase activity is required for endosomal carrier vesicle formation. *J Biol Chem* **269**, 21-24.

Clark E. A., Golub T. R., Lander E. S., Hynes R. O. (2000). Genomic analysis of metastasis reveals an essential role for RhoC. *Nature* **406**, 532-535.

Clark M. E. & Dasgupta A. (1990). A transcriptionally active form of TFIIIC is modified in poliovirus-infected HeLa cells. *Mol Cell Biol* **10**, 5106-5113.

Clarkson N. A., Kaufman R., Lublin D. M., Ward T., Pipkin P. A., Minor P. D., Evans D. J., Almond J. W. (1995). Characterization of the echovirus 7 receptor: Domains of CD55 critical for virus binding. *J Virol* **69**, 5497-5501.

Cohen C. J., Shieh J. T., Pickles R. J., Okegawa T., Hsieh J. T., Bergelson J. M. (2001). The coxsackievirus and adenovirus receptor is a transmembrane component of the tight junction. *Proc Natl Acad Sci U S A* **98**, 15191-15196.

Colonno R. J. (1986). Cell surface receptors for picornaviruses. *Bioessays* **5**, 270-274.

Conner S. D. & Schmid S. L. (2003). Regulated portals of entry into the cell. *Nature* **422**, 37-44.

Conrad P. A., Smart E. J., Ying Y. S., Anderson R. G., Bloom G. S. (1995). Caveolin cycles between plasma membrane caveolae and the golgi complex by microtubule-dependent and microtubule-independent steps. *J Cell Biol* **131**, 1421-1433.

Cooray S. (2004). The pivotal role of phosphatidylinositol 3-kinase-akt signal transduction in virus survival. *J Gen Virol* **85**, 1065-1076.

Cordes N. & Park C. C. (2007). Beta1 integrin as a molecular therapeutic target. *Int J Radiat Biol* **83**, 753-760.

Coyne C. B. & Bergelson J. M. (2006). Virus-induced abl and fyn kinase signals permit coxsackievirus entry through epithelial tight junctions. *Cell* **124**, 119-131.

Coyne C. B., Kim K. S., Bergelson J. M. (2007a). Poliovirus entry into human brain microvascular cells requires receptor-induced activation of SHP-2. *EMBO J* **26**, 4016-4028.

Coyne C. B., Shen L., Turner J. R., Bergelson J. M. (2007b). Coxsackievirus entry across epithelial tight junctions requires occludin and the small GTPases Rab34 and Rab5. *Cell Host Microbe* **2**, 181-192.

Craig A., Fernandez-Reyes D., Mesri M., McDowall A., Altieri D. C., Hogg N., Newbold C. (2000). A functional analysis of a natural variant of intercellular adhesion molecule-1 (ICAM-1Kilifi). *Hum Mol Genet* **9**, 525-530.

Cremona O. & De Camilli P. (1997). Synaptic vesicle endocytosis. *Curr Opin Neurobiol* **7**, 323-330.

Dahlquist G., Frisk G., Ivarsson S. A., Svanberg L., Forsgren M., Diderholm H. (1995). Indications that maternal coxsackie B virus infection during pregnancy is a risk factor for childhood-onset IDDM. *Diabetologia* **38**, 1371-1373.

Dahlquist G. G., Patterson C., Soltesz G. (1999). Perinatal risk factors for childhood type 1 diabetes in europe. the EURODIAB substudy 2 study group. *Diabetes Care* **22**, 1698-1702.

Dalldorf G. & Sickles G. M. (1948). An unidentified, filtrable agent isolated from the feces of children with paralysis. *Science* **108**, 61-62.

Damjanovich L., Fulop B., Adany R., Nemes Z. (1997). Integrin expression on normal and neoplastic human breast epithelium. *Acta Chir Hung* **36**, 69-71.

Deneka M., Neeft M., Popa I., van Oort M., Sprong H., Oorschot V., Klumperman J., Schu P., van der Sluijs P. (2003). Rabaptin-5alpha/rabaptin-4 serves as a linker between rab4 and gamma(1)-adaptin in membrane recycling from endosomes. *EMBO J* **22**, 2645-2657.

Denman A. M. & Rager-Zisman B. (2004). Viruses and autoimmune diseases--adapting koch's postulates. *Autoimmun Rev* **3**, 355-361.

DeTulleo L. & Kirchhausen T. (1998). The clathrin endocytic pathway in viral infection. *EMBO J* **17**, 4585-4593.

Devendra D., Liu E., Eisenbarth G. S. (2004). Type 1 diabetes: Recent developments. *BMJ* **328**, 750-754.

Dharmawardhane S., Schurmann A., Sells M. A., Chernoff J., Schmid S. L., Bokoch G. M. (2000). Regulation of macropinocytosis by p21-activated kinase-1. *Mol Biol Cell* **11**, 3341-3352.

Dickeson S. K., Mathis N. L., Rahman M., Bergelson J. M., Santoro S. A. (1999). Determinants of ligand binding specificity of the alpha(1)beta(1) and alpha(2)beta(1) integrins. *J Biol Chem* **274**, 32182-32191.

Dickeson S. K., Bhattacharyya-Pakrasi M., Mathis N. L., Schlesinger P. H., Santoro S. A. (1998). Ligand binding results in divalent cation displacement from the alpha 2 beta 1 integrin I domain: Evidence from terbium luminescence spectroscopy. *Biochemistry* **37**, 11280-11288.

Dietzen D. J., Hastings W. R., Lublin D. M. (1995). Caveolin is palmitoylated on multiple cysteine residues. palmitoylation is not necessary for localization of caveolin to caveolae. *J Biol Chem* **270**, 6838-6842.

Dorner A. J., Semler B. L., Jackson R. J., Hanecak R., Duprey E., Wimmer E. (1984). In vitro translation of poliovirus RNA: Utilization of internal initiation sites in reticulocyte lysate. *J Virol* **50**, 507-514.

Dreschers S., Dumitru C. A., Adams C., Gulbins E. (2007). The cold case: Are rhinoviruses perfectly adapted pathogens? *Cell Mol Life Sci* **64**, 181-191.

Dupree P., Parton R. G., Raposo G., Kurzchalia T. V., Simons K. (1993). Caveolae and sorting in the trans-golgi network of epithelial cells. *EMBO J* **12**, 1597-1605.

Egger D., Gosert R., Bienz K. (2002). Role of cellular structures in viral RNA replication. In *Molecular Biology of Picornaviruses* pp. 247-57. Edited by B.L. Semler and E. Wimmer, Washington DC: ASM Press. Washington DC: ASM Press.

Elias D., Markovits D., Reshef T., van der Zee R., Cohen I. R. (1990). Induction and therapy of autoimmune diabetes in the non-obese diabetic (NOD/Lt) mouse by a 65-kDa heat shock protein. *Proc Natl Acad Sci U S A* **87**, 1576-1580.

Elliott B. E., Ekblom P., Pross H., Niemann A., Rubin K. (1994). Anti-beta 1 integrin IgG inhibits pulmonary macrometastasis and the size of micrometastases from a murine mammary carcinoma. *Cell Adhes Commun* **1**, 319-332.

Elshebani A., Olsson A., Westman J., Tuvemo T., Korsgren O., Frisk G. (2007). Effects on isolated human pancreatic islet cells after infection with strains of enterovirus isolated at clinical presentation of type 1 diabetes. *Virus Res* **124**, 193-203.

Empig C. J. & Goldsmith M. A. (2002). Association of the caveola vesicular system with cellular entry by filoviruses. *J Virol* **76**, 5266-5270.

Emsley J., Knight C. G., Farndale R. W., Barnes M. J., Liddington R. C. (2000). Structural basis of collagen recognition by integrin alpha2beta1. *Cell* **101**, 47-56.

Engelman J. A., Wykoff C. C., Yasuhara S., Song K. S., Okamoto T., Lisanti M. P. (1997). Recombinant expression of caveolin-1 in oncogenically transformed cells abrogates anchorage-independent growth. *J Biol Chem* **272**, 16374-16381.

Escribano-Romero E., Jimenez-Clavero M. A., Gomes P., Garcia-Ranea J. A., Ley V. (2004). Heparan sulphate mediates swine vesicular disease virus attachment to the host cell. *J Gen Virol* **85**, 653-663.

Esfandiarei M., Luo H., Yanagawa B., Suarez A., Dabiri D., Zhang J., McManus B. M. (2004). Protein kinase B/Akt regulates coxsackievirus B3 replication through a mechanism which is not caspase dependent. *J Virol* **78**, 4289-4298.

Etchison D., Milburn S. C., Edery I., Sonenberg N., Hershey J. W. (1982). Inhibition of HeLa cell protein synthesis following poliovirus infection correlates with the proteolysis of a 220,000-dalton polypeptide associated with eucaryotic initiation factor 3 and a cap binding protein complex. *J Biol Chem* **257**, 14806-14810.

Evans D. J. & Almond J. W. (1998). Cell receptors for picornaviruses as determinants of cell tropism and pathogenesis. *Trends Microbiol* **6**, 198-202.

Everett H. & McFadden G. (2001). Viruses and apoptosis: Meddling with mitochondria. *Virology* **288**, 1-7.

Falk M. M., Grigera P. R., Bergmann I. E., Zibert A., Multhaup G., Beck E. (1990). Foot-and-mouth disease virus protease 3C induces specific proteolytic cleavage of host cell histone H3. *J Virol* **64**, 748-756.

Fan G. H., Lapierre L. A., Goldenring J. R., Richmond A. (2003). Differential regulation of CXCR2 trafficking by rab GTPases. *Blood* **101**, 2115-2124.

Fastenberg M. E., Shogomori H., Xu X., Brown D. A., London E. (2003). Exclusion of a transmembrane-type peptide from ordered-lipid domains (rafts) detected by fluorescence quenching: Extension of quenching analysis to account for the effects of domain size and domain boundaries. *Biochemistry* **42**, 12376-12390.

Feire A. L., Koss H., Compton T. (2004). Cellular integrins function as entry receptors for human cytomegalovirus via a highly conserved disintegrin-like domain. *Proc Natl Acad Sci U S A* **101**, 15470-15475.

Felding-Habermann B., O'Toole T. E., Smith J. W., Fransvea E., Ruggeri Z. M., Ginsberg M. H., Hughes P. E., Pampori N., Shattil S. J., Saven A., Mueller B. M. (2001). Integrin activation controls metastasis in human breast cancer. *Proc Natl Acad Sci U S A* **98**, 1853-1858.

Feng Y., Press B., Wandinger-Ness A. (1995). Rab 7: An important regulator of late endocytic membrane traffic. *J Cell Biol* **131**, 1435-1452.

Fernandez-Reyes D., Craig A. G., Kyes S. A., Peshu N., Snow R. W., Berendt A. R., Marsh K., Newbold C. I. (1997). A high frequency african coding polymorphism in the N-terminal domain of ICAM-1 predisposing to cerebral malaria in kenya. *Hum Mol Genet* **6**, 1357-1360.

Finley D., Ozkaynak E., Varshavsky A. (1987). The yeast polyubiquitin gene is essential for resistance to high temperatures, starvation, and other stresses. *Cell* **48**, 1035-1046.

Fogli A., Guerrini R., Moro F., Fernandez-Alvarez E., Livet M. O., Renieri A., Cioni M., Pilz D. T., Veggiotti P.& other authors. (1999). Intracellular levels of the LIS1 protein correlate with clinical and neuroradiological findings in patients with classical lissencephaly. *Ann Neurol* **45**, 154-161.

Fox G., Parry N. R., Barnett P. V., McGinn B., Rowlands D. J., Brown F. (1989). The cell attachment site on foot-and-mouth disease virus includes the amino acid sequence RGD (arginine-glycine-aspartic acid). *J Gen Virol* **70 (Pt 3)**, 625-637.

Fra A. M., Masserini M., Palestini P., Sonnino S., Simons K. (1995). A photo-reactive derivative of ganglioside GM1 specifically cross-links VIP21-caveolin on the cell surface. *FEBS Lett* **375**, 11-14.

Frick M., Bright N. A., Riento K., Bray A., Merrified C., Nichols B. J. (2007). Coassembly of flotillins induces formation of membrane microdomains, membrane curvature, and vesicle budding. *Curr Biol* **17**, 1151-1156.

Fry A. E., Auburn S., Diakite M., Green A., Richardson A., Wilson J., Jallow M., Sisay-Joof F., Pinder M.& other authors. (2008). Variation in the ICAM1 gene is not associated with severe malaria phenotypes. *Genes Immun* **9**, 462-469.

Fuchs A., Cella M., Giurisato E., Shaw A. S., Colonna M. (2004). Cutting edge: CD96 (tactile) promotes NK cell-target cell adhesion by interacting with the poliovirus receptor (CD155). *J Immunol* **172**, 3994-3998.

Fujita S., Watanabe M., Kubota T., Teramoto T., Kitajima M. (1995). Alteration of expression in integrin beta 1-subunit correlates with invasion and metastasis in colorectal cancer. *Cancer Lett* **91**, 145-149.

Furger K. A., Allan A. L., Wilson S. M., Hota C., Vantyghem S. A., Postenka C. O., Al-Katib W., Chambers A. F., Tuck A. B. (2003). Beta(3) integrin expression increases breast carcinoma cell responsiveness to the malignancy-enhancing effects of osteopontin. *Mol Cancer Res* **1**, 810-819.

Fuxe J., Liu L., Malin S., Philipson L., Collins V. P., Pettersson R. F. (2003). Expression of the coxsackie and adenovirus receptor in human astrocytic tumors and xenografts. *Int J Cancer* **103**, 723-729.

Gagescu R., Demaurex N., Parton R. G., Hunziker W., Huber L. A., Gruenberg J. (2000). The recycling endosome of madin-darby canine kidney cells is a mildly acidic compartment rich in raft components. *Mol Biol Cell* **11**, 2775-2791.

Gahmberg C. G., Fagerholm S. C., Nurmi S. M., Chavakis T., Marchesan S., Gronholm M. (2009). Regulation of integrin activity and signalling. *Biochim Biophys Acta* **1790**, 431-444.

Galbiati F., Engelman J. A., Volonte D., Zhang X. L., Minetti C., Li M., Hou H.,Jr, Kneitz B., Edelmann W., Lisanti M. P. (2001). Caveolin-3 null mice show a loss of caveolae, changes in the microdomain distribution of the dystrophin-glycoprotein complex, and t-tubule abnormalities. *J Biol Chem* **276**, 21425-21433.

Gamble D. R., Kinsley M. L., FitzGerald M. G., Bolton R., Taylor K. W. (1969). Viral antibodies in diabetes mellitus. *Br Med J* **3**, 627-630.

Garcia-Ranea J. A. & Valencia A. (1998). Distribution and functional diversification of the ras superfamily in saccharomyces cerevisiae. *FEBS Lett* **434**, 219-225.

Giancotti F. G. (2000). Complexity and specificity of integrin signalling. *Nat Cell Biol* **2**, E13-4.

Giannone G., Dubin-Thaler B. J., Dobereiner H. G., Kieffer N., Bresnick A. R., Sheetz M. P. (2004). Periodic lamellipodial contractions correlate with rearward actin waves. *Cell* **116**, 431-443.

Gibbon C., Smith T., Egger P., Betts P., Phillips D. (1997). Early infection and subsequent insulin dependent diabetes. *Arch Dis Child* **77**, 384-385.

Gingras A. C., Kennedy S. G., O'Leary M. A., Sonenberg N., Hay N. (1998). 4E-BP1, a repressor of mRNA translation, is phosphorylated and inactivated by the akt(PKB) signaling pathway. *Genes Dev* **12**, 502-513.

Glasgow G. M., McGee M. M., Sheahan B. J., Atkins G. J. (1997). Death mechanisms in cultured cells infected by semliki forest virus. *J Gen Virol* **78 (Pt 7)**, 1559-1563.

Glebov O. O., Bright N. A., Nichols B. J. (2006). Flotillin-1 defines a clathrin-independent endocytic pathway in mammalian cells. *Nat Cell Biol* **8**, 46-54.

Glukhova M., Koteliansky V., Sastre X., Thiery J. P. (1995). Adhesion systems in normal breast and in invasive breast carcinoma. *Am J Pathol* **146**, 706-716.

Goel H. L. & Languino L. R. (2004). Integrin signaling in cancer. *Cancer Treat Res* **119**, 15-31.

Gombos I., Detre C., Vamosi G., Matko J. (2004). Rafting MHC-II domains in the APC (presynaptic) plasma membrane and the thresholds for T-cell activation and immunological synapse formation. *Immunol Lett* **92**, 117-124.

Gombos I., Kiss E., Detre C., Laszlo G., Matko J. (2006). Cholesterol and sphingolipids as lipid organizers of the immune cells' plasma membrane: Their impact on the functions of MHC molecules, effector T-lymphocytes and T-cell death. *Immunol Lett* **104**, 59-69.

Gombos I., Steinbach G., Pomozi I., Balogh A., Vamosi G., Gansen A., Laszlo G., Garab G., Matko J. (2008). Some new faces of membrane microdomains: A complex confocal fluorescence, differential polarization, and FCS imaging study on live immune cells. *Cytometry A* **73**, 220-229.

Gonzalez M. A., Pinder S. E., Wencyk P. M., Bell J. A., Elston C. W., Nicholson R. I., Robertson J. F., Blamey R. W., Ellis I. O. (1999). An immunohistochemical examination of the expression of E-cadherin, alpha- and beta/gamma-catenins, and alpha2- and beta1-integrins in invasive breast cancer. *J Pathol* **187**, 523-529.

Goodfellow I. G., Sioofy A. B., Powell R. M., Evans D. J. (2001). Echoviruses bind heparan sulfate at the cell surface. *J Virol* **75**, 4918-4921.

Goodfellow I. G., Evans D. J., Blom A. M., Kerrigan D., Miners J. S., Morgan B. P., Spiller O. B. (2005). Inhibition of coxsackie B virus infection by soluble forms of its receptors: Binding affinities, altered particle formation, and competition with cellular receptors. *J Virol* **79**, 12016-12024.

Gorvel J. P., Chavrier P., Zerial M., Gruenberg J. (1991). rab5 controls early endosome fusion in vitro. *Cell* **64**, 915-925.

Gosselin A. S., Simonin Y., Guivel-Benhassine F., Rincheval V., Vayssiere J. L., Mignotte B., Colbere-Garapin F., Couderc T., Blondel B. (2003). Poliovirus-induced apoptosis is reduced in cells expressing a mutant CD155 selected during persistent poliovirus infection in neuroblastoma cells. *J Virol* **77**, 790-798.

Gradi A., Svitkin Y. V., Imataka H., Sonenberg N. (1998). Proteolysis of human eukaryotic translation initiation factor eIF4GII, but not eIF4GI, coincides with the shutoff of host protein synthesis after poliovirus infection. *Proc Natl Acad Sci U S A* **95**, 11089-11094.

Grant B. D. & Donaldson J. G. (2009). Pathways and mechanisms of endocytic recycling. *Nat Rev Mol Cell Biol* **10**, 597-608.

Greve J. M., Davis G., Meyer A. M., Forte C. P., Yost S. C., Marlor C. W., Kamarck M. E., McClelland A. (1989). The major human rhinovirus receptor is ICAM-1. *Cell* **56**, 839-847.

Grishin A. V., Azhipa O., Semenov I., Corey S. J. (2001). Interaction between growth arrest-DNA damage protein 34 and src kinase lyn negatively regulates genotoxic apoptosis. *Proc Natl Acad Sci U S A* **98**, 10172-10177.

Grist N. R., Bell E. J., Assaad F. (1978). Enteroviruses in human disease. *Prog Med Virol* **24**, 114-157.

Grunert H. P., Wolf K. U., Langner K. D., Sawitzky D., Habermehl K. O., Zeichhardt H. (1997). Internalization of human rhinovirus 14 into HeLa and ICAM-1-transfected BHK cells. *Med Microbiol Immunol* **186**, 1-9.

Gu F. & Gruenberg J. (2000). ARF1 regulates pH-dependent COP functions in the early endocytic pathway. *J Biol Chem* **275**, 8154-8160.

Gu F. & Gruenberg J. (1999). Biogenesis of transport intermediates in the endocytic pathway. *FEBS Lett* **452**, 61-66.

Guerrero C. A., Mendez E., Zarate S., Isa P., Lopez S., Arias C. F. (2000). Integrin alpha(v)beta(3) mediates rotavirus cell entry. *Proc Natl Acad Sci U S A* **97**, 14644-14649.

Guo W., Pylayeva Y., Pepe A., Yoshioka T., Muller W. J., Inghirami G., Giancotti F. G. (2006). Beta 4 integrin amplifies ErbB2 signaling to promote mammary tumorigenesis. *Cell* **126**, 489-502.

Gupta N. & DeFranco A. L. (2003). Visualizing lipid raft dynamics and early signaling events during antigen receptor-mediated B-lymphocyte activation. *Mol Biol Cell* **14**, 432-444.

Haddad A., Nokhbeh M. R., Alexander D. A., Dawe S. J., Grise C., Gulzar N., Dimock K. (2004). Binding to decay-accelerating factor is not required for infection of human leukocyte cell lines by enterovirus 70. *J Virol* **78**, 2674-2681.

Haley E. S., Au G. G., Carlton B. R., Barry R. D., Shafren D. R. (2009). Regional administration of oncolytic echovirus 1 as a novel therapy for the peritoneal dissemination of gastric cancer. *J Mol Med* **87**, 385-399.

Hall A. (1998). Rho GTPases and the actin cytoskeleton. *Science* **279**, 509-514.

Hall C. L., Dai J., van Golen K. L., Keller E. T., Long M. W. (2006). Type I collagen receptor (alpha 2 beta 1) signaling promotes the growth of human prostate cancer cells within the bone. *Cancer Res* **66**, 8648-8654.

Hall C. L., Dubyk C. W., Riesenberger T. A., Shein D., Keller E. T., van Golen K. L. (2008). Type I collagen receptor (alpha2beta1) signaling promotes prostate cancer invasion through RhoC GTPase. *Neoplasia* **10**, 797-803.

Hammond A. T., Heberle F. A., Baumgart T., Holowka D., Baird B., Feigenson G. W. (2005). Crosslinking a lipid raft component triggers liquid ordered-liquid disordered phase separation in model plasma membranes. *Proc Natl Acad Sci U S A* **102**, 6320-6325.

Hanecak R., Semler B. L., Anderson C. W., Wimmer E. (1982). Proteolytic processing of poliovirus polypeptides: Antibodies to polypeptide P3-7c inhibit cleavage at glutamine-glycine pairs. *Proc Natl Acad Sci U S A* **79**, 3973-3977.

Hao M. & Maxfield F. R. (2000). Characterization of rapid membrane internalization and recycling. *J Biol Chem* **275**, 15279-15286.

Harbison C. E., Chiorini J. A., Parrish C. R. (2008). The parvovirus capsid odyssey: From the cell surface to the nucleus. *Trends Microbiol* **16**, 208-214.

Harms J. F., Welch D. R., Samant R. S., Shevde L. A., Miele M. E., Babu G. R., Goldberg S. F., Gilman V. R., Sosnowski D. M.& other authors. (2004). A small molecule antagonist of the alpha(v)beta3 integrin suppresses MDA-MB-435 skeletal metastasis. *Clin Exp Metastasis* **21**, 119-128.

He Y., Chipman P. R., Howitt J., Bator C. M., Whitt M. A., Baker T. S., Kuhn R. J., Anderson C. W., Freimuth P., Rossmann M. G. (2001). Interaction of coxsackievirus B3 with the full length coxsackievirus-adenovirus receptor. *Nat Struct Biol* **8**, 874-878.

He Y., Lin F., Chipman P. R., Bator C. M., Baker T. S., Shoham M., Kuhn R. J., Medof M. E., Rossmann M. G. (2002). Structure of decay-accelerating factor bound to echovirus 7: A virus-receptor complex. *Proc Natl Acad Sci U S A* **99**, 10325-10329.

Hedman K., Goldenthal K. L., Rutherford A. V., Pastan I., Willingham M. C. (1987). Comparison of the intracellular pathways of transferrin recycling and vesicular stomatitis virus membrane glycoprotein exocytosis by ultrastructural double-label cytochemistry. *J Histochem Cytochem* **35**, 233-243.

Heikkilä O., Susi P., Tevaluoto T., Härmä H., Marjomäki V., Hyypiä T., Kiljunen S. (2010). Internalization of coxsackievirus A9 is mediated by {beta}2-microglobulin, dynamin and Arf6 but not caveolin-1 or clathrin. *J Virol* .

Helgason T. & Jonasson M. R. (1981). Evidence for a food additive as a cause of ketosis-prone diabetes. *Lancet* **2**, 716-720.

Hemler M. E., Jacobson J. G., Strominger J. L. (1985). Biochemical characterization of VLA-1 and VLA-2. cell surface heterodimers on activated T cells. *J Biol Chem* **260**, 15246-15252.

Herdegen T. & Leah J. D. (1998). Inducible and constitutive transcription factors in the mammalian nervous system: Control of gene expression by jun, fos and krox, and CREB/ATF proteins. *Brain Res Brain Res Rev* **28**, 370-490.

Hill M. M., Bastiani M., Luetterforst R., Kirkham M., Kirkham A., Nixon S. J., Walser P., Abankwa D., Oorschot V. M.& other authors. (2008). PTRF-cavin, a conserved cytoplasmic protein required for caveola formation and function. *Cell* **132**, 113-124.

Hiltunen M., Hyöty H., Knip M., Ilonen J., Reijonen H., Vahäsalo P., Roivainen M., Lönnrot M., Leinikki P., Hovi T., Akerblom H. K. (1997). Islet cell antibody seroconversion in children is temporally associated with enterovirus infections. childhood diabetes in finland (DiMe) study group. *J Infect Dis* **175**, 554-560.

Hirst J. & Robinson M. S. (1998). Clathrin and adaptors. *Biochim Biophys Acta* **1404**, 173-193.

Horwitz M. S., Bradley L. M., Harbertson J., Krahl T., Lee J., Sarvetnick N. (1998). Diabetes induced by coxsackie virus: Initiation by bystander damage and not molecular mimicry. *Nat Med* **4**, 781-785.

Hueffer K., Govindasamy L., Agbandje-McKenna M., Parrish C. R. (2003). Combinations of two capsid regions controlling canine host range determine canine transferrin receptor binding by canine and feline parvoviruses. *J Virol* **77**, 10099-10105.

Hughes P. J., Horsnell C., Hyypia T., Stanway G. (1995). The coxsackievirus A9 RGD motif is not essential for virus viability. *J Virol* **69**, 8035-8040.

Hullinger T. G., McCauley L. K., DeJoode M. L., Somerman M. J. (1998). Effect of bone proteins on human prostate cancer cell lines in vitro. *Prostate* **36**, 14-22.

Hultcrantz M., Huhn M. H., Wolf M., Olsson A., Jacobson S., Williams B. R., Korsgren O., Flodstrom-Tullberg M. (2007). Interferons induce an antiviral state in human pancreatic islet cells. *Virology* **367**, 92-101.

Humphries M. J. (2002). Insights into integrin-ligand binding and activation from the first crystal structure. *Arthritis Res* **4 Suppl 3**, S69-78.

Huttunen P., Heino J., Hyypia T. (1997). Echovirus 1 replication, not only virus binding to its receptor, VLA-2, is required for the induction of cellular immediate-early genes. *J Virol* **71**, 4176-4180.

Huttunen P., Hyypia T., Vihinen P., Nissinen L., Heino J. (1998). Echovirus 1 infection induces both stress- and growth-activated mitogen-activated protein kinase pathways and regulates the transcription of cellular immediate-early genes. *Virology* **250**, 85-93.

Hviid A., Stellfeld M., Wohlfahrt J., Melbye M. (2004). Childhood vaccination and type 1 diabetes. *N Engl J Med* **350**, 1398-1404.

Hwang H. Y., Kim J. Y., Lim J. Y., Chung S. K., Nam J. H., Park S. I. (2007). Coxsackievirus B3 modulates cell death by downregulating activating transcription factor 3 in HeLa cells. *Virus Res* **130**, 10-17.

Hynes R. O. (2002). Integrins: Bidirectional, allosteric signaling machines. *Cell* **110**, 673-687.

Hynes R. O. (1992). Integrins: Versatility, modulation, and signaling in cell adhesion. *Cell* **69**, 11-25.

Hynes R. O. & Lander A. D. (1992). Contact and adhesive specificities in the associations, migrations, and targeting of cells and axons. *Cell* **68**, 303-322.

Hyypiä T., Hovi T., Knowles N. J., Stanway G. (1997). Classification of enteroviruses based on molecular and biological properties. *J Gen Virol* **78 (Pt 1)**, 1-11.

Hyöty H. & Taylor K. W. (2002). The role of viruses in human diabetes. *Diabetologia* **45**, 1353-1361.

Hyöty H., Hiltunen M., Knip M., Laakkonen M., Vähäsalo P., Karjalainen J., Koskela P., Roivainen M., Leinikki P., Hovi T. (1995). A prospective study of the role of coxsackie B and other enterovirus infections in the pathogenesis of IDDM. childhood diabetes in finland (DiMe) study group. *Diabetes* **44**, 652-657.

Härkönen T., Lankinen H., Davydova B., Hovi T., Roivainen M. (2002). Enterovirus infection can induce immune responses that cross-react with beta-cell autoantigen tyrosine phosphatase IA-2/IAR. *J Med Virol* **66**, 340-350.

Härkönen T., Paananen A., Lankinen H., Hovi T., Vaarala O., Roivainen M. (2003). Enterovirus infection may induce humoral immune response reacting with islet cell autoantigens in humans. *J Med Virol* **69**, 426-440.

Ikoma T., Takahashi T., Nagano S., Li Y. M., Ohno Y., Ando K., Fujiwara T., Fujiwara H., Kosai K. (2004). A definitive role of RhoC in metastasis of orthotopic lung cancer in mice. *Clin Cancer Res* **10**, 1192-1200.

Imagawa A., Hanafusa T., Miyagawa J., Matsuzawa Y. (2000). A novel subtype of type 1 diabetes mellitus characterized by a rapid onset and an absence of diabetes-related antibodies. osaka IDDM study group. *N Engl J Med* **342**, 301-307.

Jackson T., Ellard F. M., Ghazaleh R. A., Brookes S. M., Blakemore W. E., Corteyn A. H., Stuart D. I., Newman J. W., King A. M. (1996). Efficient infection of cells in culture by type O foot-and-mouth disease virus requires binding to cell surface heparan sulfate. *J Virol* **70**, 5282-5287.

Jacobson S. J., Konings D. A., Sarnow P. (1993). Biochemical and genetic evidence for a pseudoknot structure at the 3' terminus of the poliovirus RNA genome and its role in viral RNA amplification. *J Virol* **67**, 2961-2971.

Jensen L. J., Kuhn M., Stark M., Chaffron S., Creevey C., Muller J., Doerks T., Julien P., Roth A.& other authors. (2009). STRING 8--a global view on proteins and their functional interactions in 630 organisms. *Nucleic Acids Res* **37**, D412-6.

Johannes G., Carter M. S., Eisen M. B., Brown P. O., Sarnow P. (1999). Identification of eukaryotic mRNAs that are translated at reduced cap binding complex eIF4F concentrations using a cDNA microarray. *Proc Natl Acad Sci U S A* **96**, 13118-13123.

Johnson M. S., Lu N., Denessiouk K., Heino J., Gullberg D. (2009). Integrins during evolution: Evolutionary trees and model organisms. *Biochim Biophys Acta* **1788**, 779-789.

Joki-Korpela P., Marjomäki V., Krogerus C., Heino J., Hyypiä T. (2001). Entry of human parechovirus 1. *J Virol* **75**, 1958-1967.

Jokinen J., White D. J., Salmela M., Huhtala M., Käpylä J., Sipilä K., Puranen J. S., Nissinen L., Kankaanpää P.& other authors. (2010). Molecular mechanism of alpha2beta1 integrin interaction with human echovirus 1. *EMBO J* **29**, 196-208.

Jones J. L., Critchley D. R., Walker R. A. (1992). Alteration of stromal protein and integrin expression in breast--a marker of premalignant change? *J Pathol* **167**, 399-406.

Kabouridis P. S., Magee A. I., Ley S. C. (1997). S-acylation of LCK protein tyrosine kinase is essential for its signalling function in T lymphocytes. *EMBO J* **16**, 4983-4998.

Kaila B. & Taback S. P. (2001). The effect of day care exposure on the risk of developing type 1 diabetes: A meta-analysis of case-control studies. *Diabetes Care* **24**, 1353-1358.

Kalia M., Kumari S., Chadda R., Hill M. M., Parton R. G., Mayor S. (2006). Arf6-independent GPI-anchored protein-enriched early endosomal compartments fuse with sorting endosomes via a Rab5/phosphatidylinositol-3'-kinase-dependent machinery. *Mol Biol Cell* **17**, 3689-3704.

Kaplan M. H., Klein S. W., McPhee J., Harper R. G. (1983). Group B coxsackievirus infections in infants younger than three months of age: A serious childhood illness. *Rev Infect Dis* **5**, 1019-1032.

Karjalainen M., Kakkonen E., Upla P., Paloranta H., Kankaanpää P., Liberali P., Renkema G. H., Hyypiä T., Heino J., Marjomäki V. (2008). A raft-derived, Pak1-regulated entry participates in alpha2beta1 integrin-dependent sorting to caveosomes. *Mol Biol Cell* **19**, 2857-2869.

Karlsson M. G. & Ludvigsson J. (1998). Peptide from glutamic acid decarboxylase similar to coxsackie B virus stimulates IFN-gamma mRNA expression in Th1-like lymphocytes from children with recent-onset insulin-dependent diabetes mellitus. *Acta Diabetol* **35**, 137-144.

Karnauchow T. M., Tolson D. L., Harrison B. A., Altman E., Lublin D. M., Dimock K. (1996). The HeLa cell receptor for enterovirus 70 is decay-accelerating factor (CD55). *J Virol* **70**, 5143-5152.

Kaufman D. L., Erlander M. G., Clare-Salzler M., Atkinson M. A., Maclaren N. K., Tobin A. J. (1992). Autoimmunity to two forms of glutamate decarboxylase in insulin-dependent diabetes mellitus. *J Clin Invest* **89**, 283-292.

Kaufman D. L., Clare-Salzler M., Tian J., Forsthuber T., Ting G. S., Robinson P., Atkinson M. A., Sercarz E. E., Tobin A. J., Lehmann P. V. (1993). Spontaneous loss of T-cell tolerance to glutamic acid decarboxylase in murine insulin-dependent diabetes. *Nature* **366**, 69-72.

Keller P. & Simons K. (1998). Cholesterol is required for surface transport of influenza virus hemagglutinin. *J Cell Biol* **140**, 1357-1367.

Kerekatte V., Keiper B. D., Badorff C., Cai A., Knowlton K. U., Rhoads R. E. (1999). Cleavage of poly(A)-binding protein by coxsackievirus 2A protease in vitro and in vivo: Another mechanism for host protein synthesis shutoff? *J Virol* **73**, 709-717.

Khalili P., Arakelian A., Chen G., Plunkett M. L., Beck I., Parry G. C., Donate F., Shaw D. E., Mazar A. P., Rabbani S. A. (2006). A non-RGD-based integrin binding peptide (ATN-161) blocks breast cancer growth and metastasis in vivo. *Mol Cancer Ther* **5**, 2271-2280.

Khan A. G., Pichler J., Rosemann A., Blaas D. (2007). Human rhinovirus type 54 infection via heparan sulfate is less efficient and strictly dependent on the low endosomal pH. *J Virol* .

Khan A. G., Pickl-Herk A., Gajdzig L., Marlovits T., Fuchs R., Blaas D. (2010). Human rhinovirus 14 enters rhabdomyosarcoma cells expressing ICAM-1 by a clathrin, caveolin, and flotillin-independent pathway. *J Virol* .

Kim S. M., Park J. H., Chung S. K., Kim J. Y., Hwang H. Y., Chung K. C., Jo I., Park S. I., Nam J. H. (2004). Coxsackievirus B3 infection induces cyr61 activation via JNK to mediate cell death. *J Virol* **78**, 13479-13488.

Kindberg E., Ax C., Fiore L., Svensson L. (2009). Ala67Thr mutation in the poliovirus receptor CD155 is a potential risk factor for vaccine and wild-type paralytic poliomyelitis. *J Med Virol* **81**, 933-936.

King S. L., Kamata T., Cunningham J. A., Emsley J., Liddington R. C., Takada Y., Bergelson J. M. (1997). Echovirus 1 interaction with the human very late antigen-2 (integrin alpha2beta1) I domain. identification of two independent virus contact sites distinct from the metal ion-dependent adhesion site. *J Biol Chem* **272**, 28518-28522.

Kirchhausen T. (2000). Three ways to make a vesicle. *Nat Rev Mol Cell Biol* **1**, 187-198.

Kirchhofer D., Languino L. R., Ruoslahti E., Pierschbacher M. D. (1990). Alpha 2 beta 1 integrins from different cell types show different binding specificities. *J Biol Chem* **265**, 615-618.

Knip M. & Siljander H. (2008). Autoimmune mechanisms in type 1 diabetes. *Autoimmun Rev* **7**, 550-557.

Knipe D. M., Samuel E. C., Palese P. (2001). Virus-host cell interactions. *In:Fields Virology, Eds D M Kniepe and P M Howley, Philadelphia: Lippincot Williams and Wilkins* , 133-70.

Knowlton K. U. (2008). CVB infection and mechanisms of viral cardiomyopathy. *Curr Top Microbiol Immunol* **323**, 315-335.

Kolatkar P. R., Bella J., Olson N. H., Bator C. M., Baker T. S., Rossmann M. G. (1999). Structural studies of two rhinovirus serotypes complexed with fragments of their cellular receptor. *EMBO J* **18**, 6249-6259.

Koleske A. J., Baltimore D., Lisanti M. P. (1995). Reduction of caveolin and caveolae in oncogenically transformed cells. *Proc Natl Acad Sci U S A* **92**, 1381-1385.

Kondratova A. A., Neznanov N., Kondratov R. V., Gudkov A. V. (2005). Poliovirus protein 3A binds and inactivates LIS1, causing block of membrane protein trafficking and deregulation of cell division. *Cell Cycle* **4**, 1403-1410.

Koukoulis G. K., Virtanen I., Korhonen M., Laitinen L., Quaranta V., Gould V. E. (1991). Immunohistochemical localization of integrins in the normal, hyperplastic, and neoplastic breast. correlations with their functions as receptors and cell adhesion molecules. *Am J Pathol* **139**, 787-799.

Kouranti I., Sachse M., Arouche N., Goud B., Echard A. (2006). Rab35 regulates an endocytic recycling pathway essential for the terminal steps of cytokinesis. *Curr Biol* **16**, 1719-1725.

Kozlow W. & Guise T. A. (2005). Breast cancer metastasis to bone: Mechanisms of osteolysis and implications for therapy. *J Mammary Gland Biol Neoplasia* **10**, 169-180.

Kronenberger P., Schober D., Prchla E., Ofori-Anyinam O., Vrijsen R., Rombaut B., Blaas D., Fuchs R., Boeye A. (1998). Uptake of poliovirus into the endosomal system of HeLa cells. *Arch Virol* **143**, 1417-1424.

Kruger C., Cira D., Sommer C., Fischer A., Schabitz W. R., Schneider A. (2006). Long-term gene expression changes in the cortex following cortical ischemia revealed by transcriptional profiling. *Exp Neurol* **200**, 135-152.

Kumari S. & Mayor S. (2008). ARF1 is directly involved in dynamin-independent endocytosis. *Nat Cell Biol* **10**, 30-41.

Kurozumi K., Hardcastle J., Thakur R., Shroll J., Nowicki M., Otsuki A., Chiocca E. A., Kaur B. (2008). Oncolytic HSV-1 infection of tumors induces angiogenesis and upregulates CYR61. *Mol Ther* **16**, 1382-1391.

Kurzchalia T. V., Dupree P., Parton R. G., Kellner R., Virta H., Lehnert M., Simons K. (1992). VIP21, a 21-kD membrane protein is an integral component of trans-golgi-network-derived transport vesicles. *J Cell Biol* **118**, 1003-1014.

La Linn M., Eble J. A., Lubken C., Slade R. W., Heino J., Davies J., Suhrbier A. (2005). An arthritogenic alphavirus uses the alpha1beta1 integrin collagen receptor. *Virology* **336**, 229-239.

Lafont F. & van der Goot F. G. (2005). Bacterial invasion via lipid rafts. *Cell Microbiol* **7**, 613-620.

Lahiri D. K. & Nurnberger J. I.,Jr. (1991). A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res* **19**, 5444.

Lammi N., Karvonen M., Tuomilehto J. (2005). Do microbes have a causal role in type 1 diabetes? *Med Sci Monit* **11**, RA63-9.

Lamphear B. J. & Rhoads R. E. (1996). A single amino acid change in protein synthesis initiation factor 4G renders cap-dependent translation resistant to picornaviral 2A proteases. *Biochemistry* **35**, 15726-15733.

Landsteiner K. & Popper E. (1909). Übertragung der poliomyelitis acuta auf affen in zeitschrift für immunitätsforschung und experimentelle therapie. **2**, 377-390.

Lang C., Sauter M., Szalay G., Racchi G., Grassi G., Rainaldi G., Mercatanti A., Lang F., Kandolf R., Klingel K. (2008). Connective tissue growth factor: A crucial cytokine-mediating cardiac fibrosis in ongoing enterovirus myocarditis. *J Mol Med* **86**, 49-60.

Lanzafame S., Emmanuele C., Torrisi A. (1996). Correlation of alpha 2 beta 1 integrin expression with histological type and hormonal receptor status in breast carcinomas. *Pathol Res Pract* **192**, 1031-1038.

Lanzetti L., Palamidessi A., Areces L., Scita G., Di Fiore P. P. (2004). Rab5 is a signalling GTPase involved in actin remodelling by receptor tyrosine kinases. *Nature* **429**, 309-314.

Lau C., Wang X., Song L., North M., Wiehler S., Proud D., Chow C. W. (2008). Syk associates with clathrin and mediates phosphatidylinositol 3-kinase activation during human rhinovirus internalization. *J Immunol* **180**, 870-880.

Lea S. M., Powell R. M., McKee T., Evans D. J., Brown D., Stuart D. I., van der Merwe P. A. (1998). Determination of the affinity and kinetic constants for the interaction between the human virus echovirus 11 and its cellular receptor, CD55. *J Biol Chem* **273**, 30443-30447.

Lee J. O., Rieu P., Arnaout M. A., Liddington R. (1995a). Crystal structure of the A domain from the alpha subunit of integrin CR3 (CD11b/CD18). *Cell* **80**, 631-638.

Lee J. O., Bankston L. A., Arnaout M. A., Liddington R. C. (1995b). Two conformations of the integrin A-domain (I-domain): A pathway for activation? *Structure* **3**, 1333-1340.

Leveque N., Norder H., Zreik Y., Cartet G., Falcon D., Rivat N., Chomel J. J., Hong S. S., Lina B. (2007). Echovirus 6 strains derived from a clinical isolate show differences in haemagglutination ability and cell entry pathway. *Virus Res* **130**, 1-9.

Li C. & Wong W. H. (2001). Model-based analysis of oligonucleotide arrays: Expression index computation and outlier detection. *Proc Natl Acad Sci U S A* **98**, 31-36.

Li C., Wang H., Shih S. R., Chen T. C., Li M. L. (2007). The efficacy of viral capsid inhibitors in human enterovirus infection and associated diseases. *Curr Med Chem* **14**, 847-856.

Liapis H., Flath A., Kitazawa S. (1996). Integrin alpha V beta 3 expression by bone-residing breast cancer metastases. *Diagn Mol Pathol* **5**, 127-135.

Liberali P., Kakkonen E., Turacchio G., Valente C., Spaar A., Perinetti G., Bockmann R. A., Corda D., Colanzi A., Marjomaki V., Luini A. (2008). The closure of Pak1-dependent macropinosomes requires the phosphorylation of CtBP1/BARS. *EMBO J* **27**, 970-981.

Liljestrom P. & Garoff H. (1991). A new generation of animal cell expression vectors based on the semliki forest virus replicon. *Biotechnology (N Y)* **9**, 1356-1361.

Lisanti M. P., Scherer P. E., Vidugiriene J., Tang Z., Hermanowski-Vosatka A., Tu Y. H., Cook R. F., Sargiacomo M. (1994). Characterization of caveolin-rich membrane domains isolated from an endothelial-rich source: Implications for human disease. *J Cell Biol* **126**, 111-126.

Lundmark R., Doherty G. J., Howes M. T., Cortese K., Vallis Y., Parton R. G., McMahon H. T. (2008). The GTPase-activating protein GRAF1 regulates the CLIC/GEEC endocytic pathway. *Curr Biol* **18**, 1802-1808.

Luo H., Yanagawa B., Zhang J., Luo Z., Zhang M., Esfandiarei M., Carthy C., Wilson J. E., Yang D., McManus B. M. (2002). Coxsackievirus B3 replication is reduced by inhibition of the extracellular signal-regulated kinase (ERK) signaling pathway. *J Virol* **76**, 3365-3373.

Lönnrot M., Salminen K., Knip M., Savola K., Kulmala P., Leinikki P., Hyypiä T., Akerblom H. K., Hyöty H. (2000). Enterovirus RNA in serum is a risk factor for beta-cell autoimmunity and clinical type 1 diabetes: A prospective study. childhood diabetes in finland (DiMe) study group. *J Med Virol* **61**, 214-220.

Maclaren N. K. & Atkinson M. A. (1997). Insulin-dependent diabetes mellitus: The hypothesis of molecular mimicry between islet cell antigens and microorganisms. *Mol Med Today* **3**, 76-83.

Madden D. R. (1995). The three-dimensional structure of peptide-MHC complexes. *Annu Rev Immunol* **13**, 587-622.

Magadan J. G., Barbieri M. A., Mesa R., Stahl P. D., Mayorga L. S. (2006). Rab22a regulates the sorting of transferrin to recycling endosomes. *Mol Cell Biol* **26**, 2595-2614.

Makihira S., Yan W., Ohno S., Kawamoto T., Fujimoto K., Okimura A., Yoshida E., Noshiro M., Hamada T., Kato Y. (1999). Enhancement of cell adhesion and spreading by a cartilage-specific noncollagenous protein, cartilage matrix protein (CMP/Matrilin-1), via integrin alpha1beta1. *J Biol Chem* **274**, 11417-11423.

Marjomäki V., Pietiäinen V., Matilainen H., Upla P., Ivaska J., Nissinen L., Reunanen H., Huttunen P., Hyypiä T., Heino J. (2002). Internalization of echovirus 1 in caveolae. *J Virol* **76**, 1856-1865.

Marlin S. D. & Springer T. A. (1987). Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antigen 1 (LFA-1). *Cell* **51**, 813-819.

Marsh M. & McMahon H. T. (1999). The structural era of endocytosis. *Science* **285**, 215-220.

Marsh M. & Pelchen-Matthews A. (2000). Endocytosis in viral replication. *Traffic* **1**, 525-532.

Matsumoto T., Wang P. Y., Ma W., Sung H. J., Matoba S., Hwang P. M. (2009). Polo-like kinases mediate cell survival in mitochondrial dysfunction. *Proc Natl Acad Sci U S A* **106**, 14542-14546.

Matsuura N., Puzon-McLaughlin W., Irie A., Morikawa Y., Kakudo K., Takada Y. (1996). Induction of experimental bone metastasis in mice by transfection of integrin alpha 4 beta 1 into tumor cells. *Am J Pathol* **148**, 55-61.

McManus B. M., Yanagawa B., Rezai N., Luo H., Taylor L., Zhang M., Yuan J., Buckley J., Triche T., Schreiner G., Yang D. (2002). Genetic determinants of coxsackievirus B3 pathogenesis. *Ann N Y Acad Sci* **975**, 169-179.

Medof M. E., Lublin D. M., Holers V. M., Ayers D. J., Getty R. R., Leykam J. F., Atkinson J. P., Tykocinski M. L. (1987). Cloning and characterization of cDNAs encoding the complete sequence of decay-accelerating factor of human complement. *Proc Natl Acad Sci U S A* **84**, 2007-2011.

Mellman I. (1996). Endocytosis and molecular sorting. *Annu Rev Cell Dev Biol* **12**, 575-625.

Mendelsohn C. L., Wimmer E., Racaniello V. R. (1989). Cellular receptor for poliovirus: Molecular cloning, nucleotide sequence, and expression of a new member of the immunoglobulin superfamily. *Cell* **56**, 855-865.

Mercer J. & Helenius A. (2009). Virus entry by macropinocytosis. *Nat Cell Biol* **11**, 510-520.

Mercer J. & Helenius A. (2008). Vaccinia virus uses macropinocytosis and apoptotic mimicry to enter host cells. *Science* **320**, 531-535.

Meresse S., Gorvel J. P., Chavrier P. (1995). The rab7 GTPase resides on a vesicular compartment connected to lysosomes. *J Cell Sci* **108 (Pt 11)**, 3349-3358.

Mettlen M., Pucadyil T., Ramachandran R., Schmid S. L. (2009). Dissecting dynamin's role in clathrin-mediated endocytosis. *Biochem Soc Trans* **37**, 1022-1026.

Milstone A. M., Petrella J., Sanchez M. D., Mahmud M., Whitbeck J. C., Bergelson J. M. (2005). Interaction with coxsackievirus and adenovirus receptor, but not with decay-accelerating factor (DAF), induces A-particle formation in a DAF-binding coxsackievirus B3 isolate. *J Virol* **79**, 655-660.

Mineo C., James G. L., Smart E. J., Anderson R. G. (1996). Localization of epidermal growth factor-stimulated Ras/Raf-1 interaction to caveolae membrane. *J Biol Chem* **271**, 11930-11935.

Mineo C., Ying Y. S., Chapline C., Jaken S., Anderson R. G. (1998). Targeting of protein kinase calpha to caveolae. *J Cell Biol* **141**, 601-610.

Miyata Y., Nishida E., Koyasu S., Yahara I., Sakai H. (1989). Protein kinase C-dependent and -independent pathways in the growth factor-induced cytoskeletal reorganization. *J Biol Chem* **264**, 15565-15568.

Monier S., Dietzen D. J., Hastings W. R., Lublin D. M., Kurzchalia T. V. (1996). Oligomerization of VIP21-caveolin in vitro is stabilized by long chain fatty acylation or cholesterol. *FEBS Lett* **388**, 143-149.

Monier S., Parton R. G., Vogel F., Behlke J., Henske A., Kurzchalia T. V. (1995). VIP21-caveolin, a membrane protein constituent of the caveolar coat, oligomerizes in vivo and in vitro. *Mol Biol Cell* **6**, 911-927.

Montesano R., Roth J., Robert A., Orci L. (1982). Non-coated membrane invaginations are involved in binding and internalization of cholera and tetanus toxins. *Nature* **296**, 651-653.

Morgan E. H. (1996). Cellular iron processing. *J Gastroenterol Hepatol* **11**, 1027-1030.

Morini M., Mottolese M., Ferrari N., Ghiorzo F., Buglioni S., Mortarini R., Noonan D. M., Natali P. G., Albini A. (2000). The alpha 3 beta 1 integrin is associated with mammary carcinoma cell metastasis, invasion, and gelatinase B (MMP-9) activity. *Int J Cancer* **87**, 336-342.

Morley S. J., Curtis P. S., Pain V. M. (1997). eIF4G: Translation's mystery factor begins to yield its secrets. *RNA* **3**, 1085-1104.

Moussad E. E. & Brigstock D. R. (2000). Connective tissue growth factor: What's in a name? *Mol Genet Metab* **71**, 276-292.

Mueller S., Cao X., Welker R., Wimmer E. (2002). Interaction of the poliovirus receptor CD155 with the dynein light chain tctex-1 and its implication for poliovirus pathogenesis. *J Biol Chem* **277**, 7897-7904.

Mukherjee S., Soe T. T., Maxfield F. R. (1999). Endocytic sorting of lipid analogues differing solely in the chemistry of their hydrophobic tails. *J Cell Biol* **144**, 1271-1284.

Myers M. A., Mackay I. R., Zimmet P. Z. (2002). A dietary cause of type 1 diabetes: Unearthing a new twist to the tale. *Diabetes Technol Ther* **4**, 193-198.

Myers M. A., Mackay I. R., Rowley M. J., Zimmet P. Z. (2001). Dietary microbial toxins and type 1 diabetes--a new meaning for seed and soil. *Diabetologia* **44**, 1199-1200.

Nash G. F., Turner L. F., Scully M. F., Kakkar A. K. (2002). Platelets and cancer. *Lancet Oncol* **3**, 425-430.

Naslavsky N., Rahajeng J., Sharma M., Jovic M., Caplan S. (2006). Interactions between EHD proteins and Rab11-FIP2: A role for EHD3 in early endosomal transport. *Mol Biol Cell* **17**, 163-177.

Nejentsev S., Sjoroos M., Soukka T., Knip M., Simell O., Lovgren T., Ilonen J. (1999). Population-based genetic screening for the estimation of type 1 diabetes mellitus risk in finland: Selective genotyping of markers in the HLA-DQB1, HLA-DQA1 and HLA-DRB1 loci. *Diabet Med* **16**, 985-992.

Newcombe N. G., Andersson P., Johansson E. S., Au G. G., Lindberg A. M., Barry R. D., Shafren D. R. (2003). Cellular receptor interactions of C-cluster human group A coxsackieviruses. *J Gen Virol* **84**, 3041-3050.

Neznanov N., Kondratova A., Chumakov K. M., Angres B., Zhumabayeva B., Agol V. I., Gudkov A. V. (2001). Poliovirus protein 3A inhibits tumor necrosis factor (TNF)-induced apoptosis by eliminating the TNF receptor from the cell surface. *J Virol* **75**, 10409-10420.

Nichols B. J. (2002). A distinct class of endosome mediates clathrin-independent endocytosis to the golgi complex. *Nat Cell Biol* **4**, 374-378.

Nicholson-Weller A., Burge J., Fearon D. T., Weller P. F., Austen K. F. (1982). Isolation of a human erythrocyte membrane glycoprotein with decay-accelerating activity for C3 convertases of the complement system. *J Immunol* **129**, 184-189.

Nieswandt B., Hafner M., Echtenacher B., Mannel D. N. (1999). Lysis of tumor cells by natural killer cells in mice is impeded by platelets. *Cancer Res* **59**, 1295-1300.

Nikkola J., Vihinen P., Vlaykova T., Hahka-Kemppinen M., Heino J., Pyrhonen S. (2004). Integrin chains beta1 and alphav as prognostic factors in human metastatic melanoma. *Melanoma Res* **14**, 29-37.

Nokhbeh M. R., Hazra S., Alexander D. A., Khan A., McAllister M., Suuronen E. J., Griffith M., Dimock K. (2005). Enterovirus 70 binds to different glycoconjugates containing alpha2,3-linked sialic acid on different cell lines. *J Virol* **79**, 7087-7094.

Nonet M. L., Holgado A. M., Brewer F., Serpe C. J., Norbeck B. A., Holleran J., Wei L., Hartwieg E., Jorgensen E. M., Alfonso A. (1999). UNC-11, a caenorhabditis elegans AP180 homologue, regulates the size and protein composition of synaptic vesicles. *Mol Biol Cell* **10**, 2343-2360.

Nordström T., Knekt M., Nordström E., Lindqvist C. (1999). A microplate-based fluorometric assay for monitoring human cancer cell attachment to cortical bone. *Anal Biochem* **267**, 37-45.

Norris J. M., Barriga K., Klingensmith G., Hoffman M., Eisenbarth G. S., Erlich H. A., Rewers M. (2003). Timing of initial cereal exposure in infancy and risk of islet autoimmunity. *JAMA* **290**, 1713-1720.

Notkins A. L., Lan M. S., Leslie R. D. (1998). IA-2 and IA-2beta: The immune response in IDDM. *Diabetes Metab Rev* **14**, 85-93.

Nykjaer A. & Willnow T. E. (2002). The low-density lipoprotein receptor gene family: A cellular swiss army knife? *Trends Cell Biol* **12**, 273-280.

Nykvist P., Tu H., Ivaska J., Käpylä J., Pihlajaniemi T., Heino J. (2000). Distinct recognition of collagen subtypes by alpha(1)beta(1) and alpha(2)beta(1) integrins. alpha(1)beta(1) mediates cell adhesion to type XIII collagen. *J Biol Chem* **275**, 8255-8261.

ODonnell V., Larocco M., Baxt B. (2008). Heparan sulfate-binding foot-and-mouth disease virus enters cells via caveola-mediated endocytosis. *J Virol* **82**, 9075-9085.

Oh J. J., Razfar A., Delgado I., Reed R. A., Malkina A., Boctor B., Slamon D. J. (2006). 3p21.3 tumor suppressor gene H37/Luca15/RBM5 inhibits growth of human lung cancer cells through cell cycle arrest and apoptosis. *Cancer Res* **66**, 3419-3427.

Ohka S., Matsuda N., Tohyama K., Oda T., Morikawa M., Kuge S., Nomoto A. (2004). Receptor (CD155)-dependent endocytosis of poliovirus and retrograde axonal transport of the endosome. *J Virol* **78**, 7186-7198.

Ohman T., King S. L., Krithivas A., Cunningham J., Dickeson S. K., Santoro S. A., Bergelson J. M. (2001). Echoviruses 1 and 8 are closely related genetically, and bind to similar determinants within the VLA-2 I domain. *Virus Res* **76**, 1-8.

Okegawa T., Pong R. C., Li Y., Bergelson J. M., Sagalowsky A. I., Hsieh J. T. (2001). The mechanism of the growth-inhibitory effect of coxsackie and adenovirus receptor (CAR) on human bladder cancer: A functional analysis of car protein structure. *Cancer Res* **61**, 6592-6600.

Oldstone M. B. (1998). Molecular mimicry and immune-mediated diseases. *FASEB J* **12**, 1255-1265.

Olson N. H., Kolatkar P. R., Oliveira M. A., Cheng R. H., Greve J. M., McClelland A., Baker T. S., Rossmann M. G. (1993). Structure of a human rhinovirus complexed with its receptor molecule. *Proc Natl Acad Sci U S A* **90**, 507-511.

Paananen A., Savolainen-Kopra C., Kaijalainen S., Vaarala O., Hovi T., Roivainen M. (2007). Genetic and phenotypic diversity of echovirus 30 strains and pathogenesis of type 1 diabetes. *J Med Virol* **79**, 945-955.

Palermo L. M., Hueffer K., Parrish C. R. (2003). Residues in the apical domain of the feline and canine transferrin receptors control host-specific binding and cell infection of canine and feline parvoviruses. *J Virol* **77**, 8915-8923.

Palmer J. P. (1987). Insulin autoantibodies: Their role in the pathogenesis of IDDM. *Diabetes Metab Rev* **3**, 1005-1015.

Parato K. A., Senger D., Forsyth P. A., Bell J. C. (2005). Recent progress in the battle between oncolytic viruses and tumours. *Nat Rev Cancer* **5**, 965-976.

Parker J. S. & Parrish C. R. (2000). Cellular uptake and infection by canine parvovirus involves rapid dynamin-regulated clathrin-mediated endocytosis, followed by slower intracellular trafficking. *J Virol* **74**, 1919-1930.

Parker J. S., Murphy W. J., Wang D., O'Brien S. J., Parrish C. R. (2001). Canine and feline parvoviruses can use human or feline transferrin receptors to bind, enter, and infect cells. *J Virol* **75**, 3896-3902.

Parrini M. C., Matsuda M., de Gunzburg J. (2005). Spatiotemporal regulation of the Pak1 kinase. *Biochem Soc Trans* **33**, 646-648.

Parton R. G., Joggerst B., Simons K. (1994). Regulated internalization of caveolae. *J Cell Biol* **127**, 1199-1215.

Patel K. P., Coyne C. B., Bergelson J. M. (2009). Dynamin- and lipid raft-dependent entry of decay-accelerating factor (DAF)-binding and non-DAF-binding coxsackieviruses into nonpolarized cells. *J Virol* **83**, 11064-11077.

Pavio N., Couderc T., Girard S., Sgro J. Y., Blondel B., Colbere-Garapin F. (2000). Expression of mutated poliovirus receptors in human neuroblastoma cells persistently infected with poliovirus. *Virology* **274**, 331-342.

Pelkmans L. (2005). Secrets of caveolae- and lipid raft-mediated endocytosis revealed by mammalian viruses. *Biochim Biophys Acta* **1746**, 295-304.

Pelkmans L. & Helenius A. (2002). Endocytosis via caveolae. *Traffic* **3**, 311-320.

Pelkmans L., Kartenbeck J., Helenius A. (2001). Caveolar endocytosis of simian virus 40 reveals a new two-step vesicular-transport pathway to the ER. *Nat Cell Biol* **3**, 473-483.

Pelkmans L., Burli T., Zerial M., Helenius A. (2004). Caveolin-stabilized membrane domains as multifunctional transport and sorting devices in endocytic membrane traffic. *Cell* **118**, 767-780.

Pelkmans L., Fava E., Grabner H., Hannus M., Habermann B., Krausz E., Zerial M. (2005). Genome-wide analysis of human kinases in clathrin- and caveolae/raft-mediated endocytosis. *Nature* **436**, 78-86.

Pelletier J. & Sonenberg N. (1989). Internal binding of eucaryotic ribosomes on poliovirus RNA: Translation in HeLa cell extracts. *J Virol* **63**, 441-444.

Pelletier J. & Sonenberg N. (1988). Internal initiation of translation of eukaryotic mRNA directed by a sequence derived from poliovirus RNA. *Nature* **334**, 320-325.

Pestova T. V. & Hellen C. U. (1999). Ribosome recruitment and scanning: What's new? *Trends Biochem Sci* **24**, 85-87.

Peters P. J., Mironov A.,Jr, Peretz D., van Donselaar E., Leclerc E., Erpel S., DeArmond S. J., Burton D. R., Williamson R. A., Vey M., Prusiner S. B. (2003). Trafficking of prion proteins through a caveolae-mediated endosomal pathway. *J Cell Biol* **162**, 703-717.

Pettigrew D. M., Williams D. T., Kerrigan D., Evans D. J., Lea S. M., Bhella D. (2006). Structural and functional insights into the interaction of echoviruses and decay-accelerating factor. *J Biol Chem* **281**, 5169-5177.

Peyruchaud O. (2007). Mechanisms of bone metastasis formation. *J Soc Biol* **201**, 229-236.

Pietiäinen V., Huttunen P., Hyypiä T. (2000). Effects of echovirus 1 infection on cellular gene expression. *Virology* **276**, 243-250.

Pietiäinen V., Marjomäki V., Upla P., Pelkmans L., Helenius A., Hyypiä T. (2004). Echovirus 1 endocytosis into caveosomes requires lipid rafts, dynamin II, and signaling events. *Mol Biol Cell* **15**, 4911-4925.

Pietiäinen V. M, Marjomäki V., Heino J., Hyypiä T. (2005). Viral entry, lipid rafts and caveosomes. *Ann Med* **37**, 394-403.

Pignatelli M., Smith M. E., Bodmer W. F. (1990). Low expression of collagen receptors in moderate and poorly differentiated colorectal adenocarcinomas. *Br J Cancer* **61**, 636-638.

Pignatelli M., Cardillo M. R., Hanby A., Stamp G. W. (1992). Integrins and their accessory adhesion molecules in mammary carcinomas: Loss of polarization in poorly differentiated tumors. *Hum Pathol* **23**, 1159-1166.

Pike L. J. (2006). Rafts defined: A report on the keystone symposium on lipid rafts and cell function. *J Lipid Res* **47**, 1597-1598.

Pille J. Y., Denoyelle C., Varet J., Bertrand J. R., Soria J., Opolon P., Lu H., Pritchard L. L., Vannier J. P.& other authors. (2005). Anti-RhoA and anti-RhoC siRNAs inhibit the proliferation and invasiveness of MDA-MB-231 breast cancer cells in vitro and in vivo. *Mol Ther* **11**, 267-274.

Platenik J., Kuramoto N., Yoneda Y. (2000). Molecular mechanisms associated with long-term consolidation of the NMDA signals. *Life Sci* **67**, 335-364.

Pontier S. M. & Muller W. J. (2008). Integrins in breast cancer dormancy. *APMIS* **116**, 677-684.

Popova S. N., Lundgren-Akerlund E., Wiig H., Gullberg D. (2007). Physiology and pathology of collagen receptors. *Acta Physiol (Oxf)* **190**, 179-187.

Powell R. M., Ward T., Goodfellow I., Almond J. W., Evans D. J. (1999). Mapping the binding domains on decay accelerating factor (DAF) for haemagglutinating enteroviruses: Implications for the evolution of a DAF-binding phenotype. *J Gen Virol* **80 (Pt 12)**, 3145-3152.

Powell R. M., Schmitt V., Ward T., Goodfellow I., Evans D. J., Almond J. W. (1998). Characterization of echoviruses that bind decay accelerating factor (CD55): Evidence that some haemagglutinating strains use more than one cellular receptor. *J Gen Virol* **79 (Pt 7)**, 1707-1713.

Press B., Feng Y., Hoflack B., Wandinger-Ness A. (1998). Mutant Rab7 causes the accumulation of cathepsin D and cation-independent mannose 6-phosphate receptor in an early endocytic compartment. *J Cell Biol* **140**, 1075-1089.

Pulli T., Koivunen E., Hyypiä T. (1997). Cell-surface interactions of echovirus 22. *J Biol Chem* **272**, 21176-21180.

Pulli T., Koskimies P., Hyypiä T. (1995). Molecular comparison of coxsackie A virus serotypes. *Virology* **212**, 30-38.

Pundziute-Lycka A., Urbonaite B., Dahlquist G. (2000). Infections and risk of type I (insulin-dependent) diabetes mellitus in lithuanian children. *Diabetologia* **43**, 1229-1234.

Qian Z. M., Tang P. L., Wang Q. (1997). Iron crosses the endosomal membrane by a carrier-mediated process. *Prog Biophys Mol Biol* **67**, 1-15.

Racaniello V. R. (2001). Picornaviridae: The viruses and their replication. in: Filds virology, eds. D.M. knipe and P.M. howley, philadelphia:Lipincot williams and wilkins. **72**, 685-722.

Rajendran L., Masilamani M., Solomon S., Tikkanen R., Stuermer C. A., Plattner H., Illges H. (2003). Asymmetric localization of flotillins/reggies in preassembled platforms confers inherent polarity to hematopoietic cells. *Proc Natl Acad Sci U S A* **100**, 8241-8246.

Rechsteiner M., Hoffman L., Dubiel W. (1993). The multicatalytic and 26 S proteases. *J Biol Chem* **268**, 6065-6068.

Reddi H. V. & Lipton H. L. (2002). Heparan sulfate mediates infection of high-neurovirulence theiler's viruses. *J Virol* **76**, 8400-8407.

Reymond N., Imbert A. M., Devilard E., Fabre S., Chabannon C., Xerri L., Farnarier C., Cantoni C., Bottino C.& other authors. (2004). DNAM-1 and PVR regulate monocyte migration through endothelial junctions. *J Exp Med* **199**, 1331-1341.

Richardson S. J., Willcox A., Bone A. J., Foulis A. K., Morgan N. G. (2009). The prevalence of enteroviral capsid protein vp1 immunostaining in pancreatic islets in human type 1 diabetes. *Diabetologia* **52**, 1143-1151.

Richer M. J. & Horwitz M. S. (2009). Coxsackievirus infection as an environmental factor in the etiology of type 1 diabetes. *Autoimmun Rev* **8**, 611-615.

Ridley A. J. (2001). Rho GTPases and cell migration. *J Cell Sci* **114**, 2713-2722.

Ridley A. J., Schwartz M. A., Burridge K., Firtel R. A., Ginsberg M. H., Borisy G., Parsons J. T., Horwitz A. R. (2003). Cell migration: Integrating signals from front to back. *Science* **302**, 1704-1709.

Rieder E., Berinstein A., Baxt B., Kang A., Mason P. W. (1996). Propagation of an attenuated virus by design: Engineering a novel receptor for a noninfectious foot-and-mouth disease virus. *Proc Natl Acad Sci U S A* **93**, 10428-10433.

Riikonen T., Westermarck J., Koivisto L., Broberg A., Kahari V. M., Heino J. (1995). Integrin alpha 2 beta 1 is a positive regulator of collagenase (MMP-1) and collagen alpha 1(I) gene expression. *J Biol Chem* **270**, 13548-13552.

Roep B. O., Hiemstra H. S., Schloot N. C., De Vries R. R., Chaudhuri A., Behan P. O., Drijfhout J. W. (2002). Molecular mimicry in type 1 diabetes: Immune cross-reactivity between islet autoantigen and human cytomegalovirus but not coxsackie virus. *Ann N Y Acad Sci* **958**, 163-165.

Roivainen M. (2006). Enteroviruses: New findings on the role of enteroviruses in type 1 diabetes. *Int J Biochem Cell Biol* **38**, 721-725.

Roivainen M. & Klingel K. (2009). Role of enteroviruses in the pathogenesis of type 1 diabetes. *Diabetologia* **52**, 995-996.

Roivainen M., Piirainen L., Hovi T. (1996). Efficient RGD-independent entry process of coxsackievirus A9. *Arch Virol* **141**, 1909-1919.

Roivainen M., Ylipaasto P., Savolainen C., Galama J., Hovi T., Otonkoski T. (2002). Functional impairment and killing of human beta cells by enteroviruses: The capacity is shared by a wide range of serotypes, but the extent is a characteristic of individual virus strains. *Diabetologia* **45**, 693-702.

Roivainen M., Hyypiä T., Piirainen L., Kalkkinen N., Stanway G., Hovi T. (1991). RGD-dependent entry of coxsackievirus A9 into host cells and its bypass after cleavage of VP1 protein by intestinal proteases. *J Virol* **65**, 4735-4740.

Roivainen M., Piirainen L., Hovi T., Virtanen I., Riikonen T., Heino J., Hyypiä T. (1994). Entry of coxsackievirus A9 into host cells: Specific interactions with alpha v beta 3 integrin, the vitronectin receptor. *Virology* **203**, 357-365.

Roivainen M., Knip M., Hyöty H., Kulmala P., Hiltunen M., Vähäsalo P., Hovi T., Akerblom H. K. (1998). Several different enterovirus serotypes can be associated with prediabetic autoimmune episodes and onset of overt IDDM. childhood diabetes in finland (DiMe) study group. *J Med Virol* **56**, 74-78.

Roivainen M., Rasilainen S., Ylipaasto P., Nissinen R., Ustinov J., Bouwens L., Eizirik D. L., Hovi T., Otonkoski T. (2000). Mechanisms of coxsackievirus-induced damage to human pancreatic beta-cells. *J Clin Endocrinol Metab* **85**, 432-440.

Rolli M., Fransvea E., Pilch J., Saven A., Felding-Habermann B. (2003). Activated integrin alphavbeta3 cooperates with metalloproteinase MMP-9 in regulating migration of metastatic breast cancer cells. *Proc Natl Acad Sci U S A* **100**, 9482-9487.

Rose D. M., Alon R., Ginsberg M. H. (2007). Integrin modulation and signaling in leukocyte adhesion and migration. *Immunol Rev* **218**, 126-134.

Rossmann M. G. (1994). Viral cell recognition and entry. *Protein Sci* **3**, 1712-1725.

Rossmann M. G., He Y., Kuhn R. J. (2002). Picornavirus-receptor interactions. *Trends Microbiol* **10**, 324-331.

Rothman J. E. & Warren G. (1994). Implications of the SNARE hypothesis for intracellular membrane topology and dynamics. *Curr Biol* **4**, 220-233.

Ruiz-Saenz J., Goez Y., Tabares W., Lopez-Herrera A. (2009). Cellular receptors for foot and mouth disease virus. *Intervirology* **52**, 201-212.

Ruoslahti E. & Pierschbacher M. D. (1987). New perspectives in cell adhesion: RGD and integrins. *Science* **238**, 491-497.

Sabharanjak S., Sharma P., Parton R. G., Mayor S. (2002). GPI-anchored proteins are delivered to recycling endosomes via a distinct cdc42-regulated, clathrin-independent pinocytic pathway. *Dev Cell* **2**, 411-423.

Sa-Carvalho D., Rieder E., Baxt B., Rodarte R., Tanuri A., Mason P. W. (1997). Tissue culture adaptation of foot-and-mouth disease virus selects viruses that bind to heparin and are attenuated in cattle. *J Virol* **71**, 5115-5123.

Sachs A. B., Sarnow P., Hentze M. W. (1997). Starting at the beginning, middle, and end: Translation initiation in eukaryotes. *Cell* **89**, 831-838.

Sachs M. D., Rauen K. A., Ramamurthy M., Dodson J. L., De Marzo A. M., Putzi M. J., Schoenberg M. P., Rodriguez R. (2002). Integrin alpha(v) and coxsackie adenovirus receptor expression in clinical bladder cancer. *Urology* **60**, 531-536.

Santti J., Hyypiä T., Halonen P. (1997). Comparison of PCR primer pairs in the detection of human rhinoviruses in nasopharyngeal aspirates. *J Virol Methods* **66**, 139-147.

Sargiacomo M., Sudol M., Tang Z., Lisanti M. P. (1993). Signal transducing molecules and glycosyl-phosphatidylinositol-linked proteins form a caveolin-rich insoluble complex in MDCK cells. *J Cell Biol* **122**, 789-807.

Saunderson R., Yu B., Trent R. J., Pamphlett R. (2004). A polymorphism in the poliovirus receptor gene differs in motor neuron disease. *Neuroreport* **15**, 383-386.

Scallan M. F., Allsopp T. E., Fazakerley J. K. (1997). Bcl-2 acts early to restrict semliki forest virus replication and delays virus-induced programmed cell death. *J Virol* **71**, 1583-1590.

Scheiffele P., Verkade P., Fra A. M., Virta H., Simons K., Ikonen E. (1998). Caveolin-1 and -2 in the exocytic pathway of MDCK cells. *J Cell Biol* **140**, 795-806.

Scherer P. E., Lewis R. Y., Volonte D., Engelman J. A., Galbiati F., Couet J., Kohtz D. S., van Donselaar E., Peters P., Lisanti M. P. (1997). Cell-type and tissue-specific expression of caveolin-2. caveolins 1 and 2 co-localize and form a stable hetero-oligomeric complex in vivo. *J Biol Chem* **272**, 29337-29346.

Schimmoller F. & Riezman H. (1993). Involvement of Ypt7p, a small GTPase, in traffic from late endosome to the vacuole in yeast. *J Cell Sci* **106 (Pt 3)**, 823-830.

Schlax P. E., Zhang J., Lewis E., Planchart A., Lawson T. G. (2007). Degradation of the encephalomyocarditis virus and hepatitis A virus 3C proteases by the ubiquitin/26S proteasome system in vivo. *Virology* **360**, 350-363.

Schnitzer J. E., Allard J., Oh P. (1995). NEM inhibits transcytosis, endocytosis, and capillary permeability: Implication of caveolae fusion in endothelia. *Am J Physiol* **268**, H48-55.

Schnitzer J. E., Oh P., Pinney E., Allard J. (1994). Filipin-sensitive caveolae-mediated transport in endothelium: Reduced transcytosis, scavenger endocytosis, and capillary permeability of select macromolecules. *J Cell Biol* **127**, 1217-1232.

Schober D., Kronenberger P., Prchla E., Blaas D., Fuchs R. (1998). Major and minor receptor group human rhinoviruses penetrate from endosomes by different mechanisms. *J Virol* **72**, 1354-1364.

Schwencke C., Braun-Dullaeus R. C., Wunderlich C., Strasser R. H. (2006). Caveolae and caveolin in transmembrane signaling: Implications for human disease. *Cardiovasc Res* **70**, 42-49.

Selinka H. C., Zibert A., Wimmer E. (1992). A chimeric poliovirus/CD4 receptor confers susceptibility to poliovirus on mouse cells. *J Virol* **66**, 2523-2526.

Selinka H. C., Zibert A., Wimmer E. (1991). Poliovirus can enter and infect mammalian cells by way of an intercellular adhesion molecule 1 pathway. *Proc Natl Acad Sci U S A* **88**, 3598-3602.

Serreze D. V., Wasserfall C., Ottendorfer E. W., Stalvey M., Pierce M. A., Gauntt C., O'Donnell B., Flanagan J. B., Campbell-Thompson M., Ellis T. M., Atkinson M. A. (2005). Diabetes acceleration or prevention by a coxsackievirus B4 infection: Critical requirements for both interleukin-4 and gamma interferon. *J Virol* **79**, 1045-1052.

Seth S., Maier M. K., Qiu Q., Ravens I., Kremmer E., Forster R., Bernhardt G. (2007). The murine pan T cell marker CD96 is an adhesion receptor for CD155 and nectin-1. *Biochem Biophys Res Commun* **364**, 959-965.

Shafren D. R., Sylvester D., Johansson E. S., Campbell I. G., Barry R. D. (2005). Oncolysis of human ovarian cancers by echovirus type 1. *Int J Cancer* **115**, 320-328.

Shafren D. R., Dorahy D. J., Greive S. J., Burns G. F., Barry R. D. (1997a). Mouse cells expressing human intercellular adhesion molecule-1 are susceptible to infection by coxsackievirus A21. *J Virol* **71**, 785-789.

Shafren D. R., Dorahy D. J., Ingham R. A., Burns G. F., Barry R. D. (1997b). Coxsackievirus A21 binds to decay-accelerating factor but requires intercellular adhesion molecule 1 for cell entry. *J Virol* **71**, 4736-4743.

Shafren D. R., Bates R. C., Agrez M. V., Herd R. L., Burns G. F., Barry R. D. (1995). Coxsackieviruses B1, B3, and B5 use decay accelerating factor as a receptor for cell attachment. *J Virol* **69**, 3873-3877.

Shafren D. R., Au G. G., Nguyen T., Newcombe N. G., Haley E. S., Beagley L., Johansson E. S., Hersey P., Barry R. D. (2004). Systemic therapy of malignant human melanoma tumors by a common cold-producing enterovirus, coxsackievirus a21. *Clin Cancer Res* **10**, 53-60.

Shaulian E. & Karin M. (2002). AP-1 as a regulator of cell life and death. *Nat Cell Biol* **4**, E131-6.

Sheff D. R., Daro E. A., Hull M., Mellman I. (1999). The receptor recycling pathway contains two distinct populations of early endosomes with different sorting functions. *J Cell Biol* **145**, 123-139.

Shevrin D. H., Gorny K. I., Rosol T. J., Kukreja S. C. (1991). Effect of etidronate disodium on the development of bone lesions in an animal model of bone metastasis using the human prostate cancer cell line PC-3. *Prostate* **19**, 149-154.

Shevrin D. H., Kukreja S. C., Ghosh L., Lad T. E. (1988). Development of skeletal metastasis by human prostate cancer in athymic nude mice. *Clin Exp Metastasis* **6**, 401-409.

Shin J. S., Gao Z., Abraham S. N. (2000). Involvement of cellular caveolae in bacterial entry into mast cells. *Science* **289**, 785-788.

Shin S. Y., Ko J., Chang J. S., Min D. S., Choi C., Bae S. S., Kim M. J., Hyun D. S., Kim J. H.& other authors. (2002). Negative regulatory role of overexpression of PLC gamma 1 in the expression of early growth response 1 gene in rat 3Y1 fibroblasts. *FASEB J* **16**, 1504-1514.

Si X., Gao G., Wong J., Wang Y., Zhang J., Luo H. (2008). Ubiquitination is required for effective replication of coxsackievirus B3. *PLoS One* **3**, e2585.

Sieczkarski S. B. & Whittaker G. R. (2005). Viral entry. *Curr Top Microbiol Immunol* **285**, 1-23.

Simons K. & Ikonen E. (1997). Functional rafts in cell membranes. *Nature* **387**, 569-572.

Simons K. & van Meer G. (1988). Lipid sorting in epithelial cells. *Biochemistry* **27**, 6197-6202.

Simonsen A., Wurmser A. E., Emr S. D., Stenmark H. (2001). The role of phosphoinositides in membrane transport. *Curr Opin Cell Biol* **13**, 485-492.

Skelding K. A., Barry R. D., Shafren D. R. (2009). Systemic targeting of metastatic human breast tumor xenografts by coxsackievirus A21. *Breast Cancer Res Treat* **113**, 21-30.

Sloan E. K., Stanley K. L., Anderson R. L. (2004). Caveolin-1 inhibits breast cancer growth and metastasis. *Oncogene* **23**, 7893-7897.

Smart E. J., Ying Y., Donzell W. C., Anderson R. G. (1996). A role for caveolin in transport of cholesterol from endoplasmic reticulum to plasma membrane. *J Biol Chem* **271**, 29427-29435.

Smith C., Estavillo D., Emsley J., Bankston L. A., Liddington R. C., Cruz M. A. (2000). Mapping the collagen-binding site in the I domain of the glycoprotein Ia/IIa (integrin alpha(2)beta(1)). *J Biol Chem* **275**, 4205-4209.

Soloaga A., Thomson S., Wiggin G. R., Rampersaud N., Dyson M. H., Hazzalin C. A., Mahadevan L. C., Arthur J. S. (2003). MSK2 and MSK1 mediate the mitogen- and stress-induced phosphorylation of histone H3 and HMG-14. *EMBO J* **22**, 2788-2797.

Springer T. A. (1997). Folding of the N-terminal, ligand-binding region of integrin alpha-subunits into a beta-propeller domain. *Proc Natl Acad Sci U S A* **94**, 65-72.

Staunton D. E., Dustin M. L., Erickson H. P., Springer T. A. (1990). The arrangement of the immunoglobulin-like domains of ICAM-1 and the binding sites for LFA-1 and rhinovirus. *Cell* **61**, 243-254.

Staunton D. E., Merluzzi V. J., Rothlein R., Barton R., Marlin S. D., Springer T. A. (1989). A cell adhesion molecule, ICAM-1, is the major surface receptor for rhinoviruses. *Cell* **56**, 849-853.

Subramaniam M., Hefferan T. E., Tau K., Peus D., Pittelkow M., Jalal S., Riggs B. L., Roche P., Spelsberg T. C. (1998). Tissue, cell type, and breast cancer stage-specific expression of a TGF-beta inducible early transcription factor gene. *J Cell Biochem* **68**, 226-236.

Suikkanen S., Sääjärvi K., Hirsimäki J., Välilehto O., Reunanen H., Vihinen-Ranta M., Vuento M. (2002). Role of recycling endosomes and lysosomes in dynein-dependent entry of canine parvovirus. *J Virol* **76**, 4401-4411.

Suwa H., Ohshio G., Imamura T., Watanabe G., Arii S., Imamura M., Narumiya S., Hiai H., Fukumoto M. (1998). Overexpression of the rhoC gene correlates with progression of ductal adenocarcinoma of the pancreas. *Br J Cancer* **77**, 147-152.

Suzuki S., Takahashi T., Nakamura S., Koike K., Ariyoshi Y., Takahashi T., Ueda R. (1993). Alterations of integrin expression in human lung cancer. *Jpn J Cancer Res* **84**, 168-174.

Syed N., Smith P., Sullivan A., Spender L. C., Dyer M., Karran L., O'Nions J., Allday M., Hoffmann I.& other authors. (2006). Transcriptional silencing of polo-like kinase 2 (SNK/PLK2) is a frequent event in B-cell malignancies. *Blood* **107**, 250-256.

Tahara-Hanaoka S., Shibuya K., Onoda Y., Zhang H., Yamazaki S., Miyamoto A., Honda S., Lanier L. L., Shibuya A. (2004). Functional characterization of DNAM-1 (CD226) interaction with its ligands PVR (CD155) and nectin-2 (PRR-2/CD112). *Int Immunol* **16**, 533-538.

Takai Y., Sasaki T., Matozaki T. (2001). Small GTP-binding proteins. *Physiol Rev* **81**, 153-208.

Tam P. E. (2006). Coxsackievirus myocarditis: Interplay between virus and host in the pathogenesis of heart disease. *Viral Immunol* **19**, 133-146.

Tanaka S., Kobayashi T., Momotsu T. (2000). A novel subtype of type 1 diabetes mellitus. *N Engl J Med* **342**, 1835-1837.

Tanaka S., Nishida Y., Aida K., Maruyama T., Shimada A., Suzuki M., Shimura H., Takizawa S., Takahashi M.& other authors. (2009). Enterovirus infection, CXC chemokine ligand 10 (CXCL10), and CXCR3 circuit: A mechanism of accelerated beta-cell failure in fulminant type 1 diabetes. *Diabetes* **58**, 2285-2291.

Thorsdottir I. & Ramel A. (2003). Dietary intake of 10- to 16-year-old children and adolescents in central and northern europe and association with the incidence of type 1 diabetes. *Ann Nutr Metab* **47**, 267-275.

Thyss R., Virolle V., Imbert V., Peyron J. F., Aberdam D., Virolle T. (2005). NF-kappaB/Egr-1/Gadd45 are sequentially activated upon UVB irradiation to mediate epidermal cell death. *EMBO J* **24**, 128-137.

Tomko R. P., Xu R., Philipson L. (1997). HCAR and MCAR: The human and mouse cellular receptors for subgroup C adenoviruses and group B coxsackieviruses. *Proc Natl Acad Sci U S A* **94**, 3352-3356.

Toyoda H., Nicklin M. J., Murray M. G., Anderson C. W., Dunn J. J., Studier F. W., Wimmer E. (1986). A second virus-encoded proteinase involved in proteolytic processing of poliovirus polyprotein. *Cell* **45**, 761-770.

Triantafilou K. & Triantafilou M. (2004). Lipid-raft-dependent coxsackievirus B4 internalization and rapid targeting to the golgi. *Virology* **326**, 6-19.

Triantafilou K. & Triantafilou M. (2003). Lipid raft microdomains: Key sites for coxsackievirus A9 infectious cycle. *Virology* **317**, 128-135.

Triantafilou K., Fradelizi D., Wilson K., Triantafilou M. (2002). GRP78, a coreceptor for coxsackievirus A9, interacts with major histocompatibility complex class I molecules which mediate virus internalization. *J Virol* **76**, 633-643.

Triantafilou M., Triantafilou K., Wilson K. M., Takada Y., Fernandez N., Stanway G. (1999). Involvement of beta2-microglobulin and integrin alphavbeta3 molecules in the coxsackievirus A9 infectious cycle. *J Gen Virol* **80 (Pt 10)**, 2591-2600.

Tuck A. B., Elliott B. E., Hota C., Tremblay E., Chambers A. F. (2000). Osteopontin-induced, integrin-dependent migration of human mammary epithelial cells involves activation of the hepatocyte growth factor receptor (met). *J Cell Biochem* **78**, 465-475.

Tulla M., Pentikäinen O. T., Viitasalo T., Käpylä J., Impola U., Nykvist P., Nissinen L., Johnson M. S., Heino J. (2001). Selective binding of collagen subtypes by integrin alpha 1I, alpha 2I, and alpha 10I domains. *J Biol Chem* **276**, 48206-48212.

Tumova S., Woods A., Couchman J. R. (2000). Heparan sulfate proteoglycans on the cell surface: Versatile coordinators of cellular functions. *Int J Biochem Cell Biol* **32**, 269-288.

Tung W. H., Sun C. C., Hsieh H. L., Wang S. W., Horng J. T., Yang C. M. (2007). EV71 induces VCAM-1 expression via PDGF receptor, PI3-K/Akt, p38 MAPK, JNK and NF-kappaB in vascular smooth muscle cells. *Cell Signal* **19**, 2127-2137.

Ulmer T. S., Calderwood D. A., Ginsberg M. H., Campbell I. D. (2003). Domain-specific interactions of talin with the membrane-proximal region of the integrin beta3 subunit. *Biochemistry* **42**, 8307-8312.

Uncapher C. R., DeWitt C. M., Colonno R. J. (1991). The major and minor group receptor families contain all but one human rhinovirus serotype. *Virology* **180**, 814-817.

Upla P., Marjomäki V., Hyypiä T. (2009). Role of lipid rafts in virus infection. *Future Virology* **4**, 487-500.

Upla P., Marjomäki V., Kankaanpää P., Ivaska J., Hyypiä T., Van Der Goot F. G., Heino J. (2004). Clustering induces a lateral redistribution of alpha 2 beta 1 integrin from membrane rafts to caveolae and subsequent protein kinase C-dependent internalization. *Mol Biol Cell* **15**, 625-636.

Vaarala O., Knip M., Paronen J., Hämäläinen A. M., Muona P., Väätäinen M., Ilonen J., Simell O., Akerblom H. K. (1999). Cow's milk formula feeding induces primary immunization to insulin in infants at genetic risk for type 1 diabetes. *Diabetes* **48**, 1389-1394.

Valdes A. M., Thomson G., Barcellos L. F. (2010). Genetic variation within the HLA class III influences T1D susceptibility conferred by high-risk HLA haplotypes. *Genes Immun* .

Van Aelst L. & D'Souza-Schorey C. (1997). Rho GTPases and signaling networks. *Genes Dev* **11**, 2295-2322.

van der Pluijm G., Vloedgraven H., van Beek E., van der Wee-Pals L., Lowik C., Papapoulos S. (1996a). Bisphosphonates inhibit the adhesion of breast cancer cells to bone matrices in vitro. *J Clin Invest* **98**, 698-705.

van der Pluijm G., Vloedgraven H. J., Ivanov B., Robey F. A., Grzesik W. J., Robey P. G., Papapoulos S. E., Lowik C. W. (1996b). Bone sialoprotein peptides are potent inhibitors of breast cancer cell adhesion to bone. *Cancer Res* **56**, 1948-1955.

van der Pluijm G., Vloedgraven H., Papapoulos S., Lowick C., Grzesik W., Kerr J., Robey P. G. (1997). Attachment characteristics and involvement of integrins in adhesion of breast cancer cell lines to extracellular bone matrix components. *Lab Invest* **77**, 665-675.

Van der Velde-Zimmermann D., Verdaasdonk M. A., Rademakers L. H., De Weger R. A., Van den Tweel J. G., Joling P. (1997). Fibronectin distribution in human bone marrow stroma: Matrix assembly and tumor cell adhesion via alpha5 beta1 integrin. *Exp Cell Res* **230**, 111-120.

van Duyl B. Y., Rijkers D. T., de Kruijff B., Killian J. A. (2002). Influence of hydrophobic mismatch and palmitoylation on the association of transmembrane alpha-helical peptides with detergent-resistant membranes. *FEBS Lett* **523**, 79-84.

van Golen K. L., Bao L., DiVito M. M., Wu Z., Prendergast G. C., Merajver S. D. (2002). Reversion of RhoC GTPase-induced inflammatory breast cancer phenotype by treatment with a farnesyl transferase inhibitor. *Mol Cancer Ther* **1**, 575-583.

van Kuppeveld F. J., de Jong A. S., Melchers W. J., Willems P. H. (2005). Enterovirus protein 2B po(u)res out the calcium: A viral strategy to survive? *Trends Microbiol* **13**, 41-44.

Varela-Calvino R. & Peakman M. (2003). Enteroviruses and type 1 diabetes. *Diabetes Metab Res Rev* **19**, 431-441.

Vihinen P., Riikonen T., Laine A., Heino J. (1996). Integrin alpha 2 beta 1 in tumorigenic human osteosarcoma cell lines regulates cell adhesion, migration, and invasion by interaction with type I collagen. *Cell Growth Differ* **7**, 439-447.

Vinogradova O., Velyvis A., Velyviene A., Hu B., Haas T., Plow E., Qin J. (2002). A structural mechanism of integrin alpha(IIb)beta(3) "inside-out" activation as regulated by its cytoplasmic face. *Cell* **110**, 587-597.

Virtanen S. M., Läärä E., Hyppönen E., Reijonen H., Räsänen L., Aro A., Knip M., Ilonen J., Akerblom H. K. (2000). Cow's milk consumption, HLA-DQB1 genotype, and type 1 diabetes: A nested case-control study of siblings of children with diabetes. childhood diabetes in finland study group. *Diabetes* **49**, 912-917.

Viskari H., Ludvigsson J., Uibo R., Salur L., Marciulionyte D., Hermann R., Soltesz G., Fuchtenbusch M., Ziegler A. G.& other authors. (2004). Relationship between the incidence of type 1 diabetes and enterovirus infections in different european populations: Results from the EPIVIR project. *J Med Virol* **72**, 610-617.

Viskari H., Ludvigsson J., Uibo R., Salur L., Marciulionyte D., Hermann R., Soltesz G., Fuchtenbusch M., Ziegler A. G.& other authors. (2005). Relationship between the incidence of type 1 diabetes and maternal enterovirus antibodies: Time trends and geographical variation. *Diabetologia* **48**, 1280-1287.

Vlahakis N. E., Young B. A., Atakilit A., Hawkridge A. E., Issaka R. B., Boudreau N., Sheppard D. (2007). Integrin alpha9beta1 directly binds to vascular endothelial growth factor (VEGF)-A and contributes to VEGF-A-induced angiogenesis. *J Biol Chem* **282**, 15187-15196.

Vlasak M., Roivainen M., Reithmayer M., Goesler I., Laine P., Snyers L., Hovi T., Blaas D. (2005). The minor receptor group of human rhinovirus (HRV) includes HRV23 and HRV25, but the presence of a lysine in the VP1 HI loop is not sufficient for receptor binding. *J Virol* **79**, 7389-7395.

Voges D., Zwickl P., Baumeister W. (1999). The 26S proteasome: A molecular machine designed for controlled proteolysis. *Annu Rev Biochem* **68**, 1015-1068.

Vonderheit A. & Helenius A. (2005). Rab7 associates with early endosomes to mediate sorting and transport of semliki forest virus to late endosomes. *PLoS Biol* **3**, e233.

Vreugdenhil G. R., Wijnands P. G., Netea M. G., van der Meer J. W., Melchers W. J., Galama J. M. (2000). Enterovirus-induced production of pro-inflammatory and T-helper cytokines by human leukocytes. *Cytokine* **12**, 1793-1796.

Vreugdenhil G. R., Geluk A., Ottenhoff T. H., Melchers W. J., Roep B. O., Galama J. M. (1998). Molecular mimicry in diabetes mellitus: The homologous domain in coxsackie B virus protein 2C and islet autoantigen GAD65 is highly conserved in the coxsackie B-like enteroviruses and binds to the diabetes associated HLA-DR3 molecule. *Diabetologia* **41**, 40-46.

Vreugdenhil G. R., Schloot N. C., Hoorens A., Rongen C., Pipeleers D. G., Melchers W. J., Roep B. O., Galama J. M. (2000). Acute onset of type I diabetes mellitus after severe echovirus 9 infection: Putative pathogenic pathways. *Clin Infect Dis* **31**, 1025-1031.

Wahab N. A., Weston B. S., Mason R. M. (2005). Modulation of the TGFbeta/Smad signaling pathway in mesangial cells by CTGF/CCN2. *Exp Cell Res* **307**, 305-314.

Wang F., Hansen R. K., Radisky D., Yoneda T., Barcellos-Hoff M. H., Petersen O. W., Turley E. A., Bissell M. J. (2002). Phenotypic reversion or death of cancer cells by altering signaling pathways in three-dimensional contexts. *J Natl Cancer Inst* **94**, 1494-1503.

Wang F., Weaver V. M., Petersen O. W., Larabell C. A., Dedhar S., Briand P., Lupu R., Bissell M. J. (1998). Reciprocal interactions between beta1-integrin and epidermal growth factor receptor in three-dimensional basement membrane breast cultures: A different perspective in epithelial biology. *Proc Natl Acad Sci U S A* **95**, 14821-14826.

Wang L. H., Rothberg K. G., Anderson R. G. (1993). Mis-assembly of clathrin lattices on endosomes reveals a regulatory switch for coated pit formation. *J Cell Biol* **123**, 1107-1117.

Ward T., Powell R. M., Pipkin P. A., Evans D. J., Minor P. D., Almond J. W. (1998). Role for beta2-microglobulin in echovirus infection of rhabdomyosarcoma cells. *J Virol* **72**, 5360-5365.

Ward T., Pipkin P. A., Clarkson N. A., Stone D. M., Minor P. D., Almond J. W. (1994). Decay-accelerating factor CD55 is identified as the receptor for echovirus 7 using CELICS, a rapid immuno-focal cloning method. *EMBO J* **13**, 5070-5074.

Way M. & Parton R. G. (1995). M-caveolin, a muscle-specific caveolin-related protein. *FEBS Lett* **376**, 108-112.

Weigert R., Yeung A. C., Li J., Donaldson J. G. (2004). Rab22a regulates the recycling of membrane proteins internalized independently of clathrin. *Mol Biol Cell* **15**, 3758-3770.

Wenzlau J. M., Juhl K., Yu L., Moua O., Sarkar S. A., Gottlieb P., Rewers M., Eisenbarth G. S., Jensen J., Davidson H. W., Hutton J. C. (2007). The cation efflux transporter ZnT8 (Slc30A8) is a major autoantigen in human type 1 diabetes. *Proc Natl Acad Sci U S A* **104**, 17040-17045.

West M. A., Bretscher M. S., Watts C. (1989). Distinct endocytotic pathways in epidermal growth factor-stimulated human carcinoma A431 cells. *J Cell Biol* **109**, 2731-2739.

White D. E. & Muller W. J. (2007). Multifaceted roles of integrins in breast cancer metastasis. *J Mammary Gland Biol Neoplasia* **12**, 135-142.

White D. J., Puranen S., Johnson M. S., Heino J. (2004). The collagen receptor subfamily of the integrins. *Int J Biochem Cell Biol* **36**, 1405-1410.

Whittaker C. A. & Hynes R. O. (2002). Distribution and evolution of von Willebrand/integrin A domains: Widely dispersed domains with roles in cell adhesion and elsewhere. *Mol Biol Cell* **13**, 3369-3387.

Wickham T. J., Mathias P., Cheresh D. A., Nemerow G. R. (1993). Integrins alpha v beta 3 and alpha v beta 5 promote adenovirus internalization but not virus attachment. *Cell* **73**, 309-319.

Williams C. H., Oikarinen S., Tauriainen S., Salminen K., Hyöty H., Stanway G. (2006). Molecular analysis of an echovirus 3 strain isolated from an individual concurrently with appearance of islet cell and IA-2 autoantibodies. *J Clin Microbiol* **44**, 441-448.

Williams C. H., Kajander T., Hyypiä T., Jackson T., Sheppard D., Stanway G. (2004). Integrin alpha v beta 6 is an RGD-dependent receptor for coxsackievirus A9. *J Virol* **78**, 6967-6973.

Willingmann P., Barnert H., Zeichhardt H., Habermehl K. O. (1989). Recovery of structurally intact and infectious poliovirus type 1 from HeLa cells during receptor-mediated endocytosis. *Virology* **168**, 417-420.

Winkles J. A. & Alberts G. F. (2005). Differential regulation of polo-like kinase 1, 2, 3, and 4 gene expression in mammalian cells and tissues. *Oncogene* **24**, 260-266.

Wolf K., Mazo I., Leung H., Engelke K., von Andrian U. H., Deryugina E. I., Strongin A. Y., Brocker E. B., Friedl P. (2003). Compensation mechanism in tumor cell migration: Mesenchymal-amoeboid transition after blocking of pericellular proteolysis. *J Cell Biol* **160**, 267-277.

Wong N. C., Mueller B. M., Barbas C. F., Ruminski P., Quaranta V., Lin E. C., Smith J. W. (1998). Alphav integrins mediate adhesion and migration of breast carcinoma cell lines. *Clin Exp Metastasis* **16**, 50-61.

Wong W. R., Chen Y. Y., Yang S. M., Chen Y. L., Horng J. T. (2005). Phosphorylation of PI3K/Akt and MAPK/ERK in an early entry step of enterovirus 71. *Life Sci* **78**, 82-90.

Woodall C. J. & Graham D. I. (2004). Evidence for neuronal localisation of enteroviral sequences in motor neurone disease/amyotrophic lateral sclerosis by in situ hybridization. *Eur J Histochem* **48**, 129-134.

Xiao C., Bator-Kelly C. M., Rieder E., Chipman P. R., Craig A., Kuhn R. J., Wimmer E., Rossmann M. G. (2005). The crystal structure of coxsackievirus A21 and its interaction with ICAM-1. *Structure* **13**, 1019-1033.

Xiao C., Tuthill T. J., Bator Kelly C. M., Challinor L. J., Chipman P. R., Killington R. A., Rowlands D. J., Craig A., Rossmann M. G. (2004). Discrimination among rhinovirus serotypes for a variant ICAM-1 receptor molecule. *J Virol* **78**, 10034-10044.

Xing L., Huhtala M., Pietiäinen V., Käpyla J., Vuorinen K., Marjomäki V., Heino J., Johnson M. S., Hyypiä T., Cheng R. H. (2004). Structural and functional analysis of integrin alpha2I domain interaction with echovirus 1. *J Biol Chem* **279**, 11632-11638.

Xiong J. P., Stehle T., Diefenbach B., Zhang R., Dunker R., Scott D. L., Joachimiak A., Goodman S. L., Arnaout M. A. (2001). Crystal structure of the extracellular segment of integrin alpha Vbeta3. *Science* **294**, 339-345.

Yalamanchili P., Harris K., Wimmer E., Dasgupta A. (1996). Inhibition of basal transcription by poliovirus: A virus- encoded protease (3Cpro) inhibits formation of TBP-TATA box complex in vitro. *J Virol* **70**, 2922-2929.

Yamayoshi S., Yamashita Y., Li J., Hanagata N., Minowa T., Takemura T., Koike S. (2009). Scavenger receptor B2 is a cellular receptor for enterovirus 71. *Nat Med* **15**, 798-801.

Yang B., Chuang H., Yang K. D. (2009). Sialylated glycans as receptor and inhibitor of enterovirus 71 infection to DLD-1 intestinal cells. *Virol J* **6**, 141.

Yang D., Yu J., Luo Z., Carthy C. M., Wilson J. E., Liu Z., McManus B. M. (1999). Viral myocarditis: Identification of five differentially expressed genes in coxsackievirus B3-infected mouse heart. *Circ Res* **84**, 704-712.

Yao E. S., Zhang H., Chen Y. Y., Lee B., Chew K., Moore D., Park C. (2007). Increased beta1 integrin is associated with decreased survival in invasive breast cancer. *Cancer Res* **67**, 659-664.

Ylipaasto P., Eskelinen M., Salmela K., Hovi T., Roivainen M. (2010). Vitronectin receptors, alpha v integrins, are recognized by several non-RGD-containing echoviruses in a continuous laboratory cell line and also in primary human langerhans' islets and endothelial cells. *J Gen Virol* **91**, 155-165.

Ylipaasto P., Eskelinen M., Salmela K., Hovi T., Roivainen M. (2009). Vitronectin receptors, {alpha}V-integrins, are recognized by several non-RGD containing echoviruses in a continuous laboratory cell line and also in primary human langerhans' islets and endothelial cells. *J Gen Virol* .

Yoon J. W., Bachurski C. J., McArthur R. G. (1986). Re: Concept of virus as an etiological agent in the development of insulin-dependent diabetes mellitus. *Diabetes Res Clin Pract* **2**, 365-366.

Yu C. C., Hsu M. J., Kuo M. L., Chen R. F., Chen M. C., Bai K. J., Yu M. C., Chen B. C., Lin C. H. (2009). Thrombin-induced connective tissue growth factor expression in human lung fibroblasts requires the ASK1/JNK/AP-1 pathway. *J Immunol* **182**, 7916-7927.

Yudowski G. A., Puthenveedu M. A., Henry A. G., von Zastrow M. (2009). Cargo-mediated regulation of a rapid Rab4-dependent recycling pathway. *Mol Biol Cell* **20**, 2774-2784.

Zamir E. & Geiger B. (2001). Molecular complexity and dynamics of cell-matrix adhesions. *J Cell Sci* **114**, 3583-3590.

Zautner A. E., Korner U., Henke A., Badorff C., Schmidtke M. (2003). Heparan sulfates and coxsackievirus-adenovirus receptor: Each one mediates coxsackievirus B3 PD infection. *J Virol* **77**, 10071-10077.

Zhang B., Koh Y. H., Beckstead R. B., Budnik V., Ganetzky B., Bellen H. J. (1998). Synaptic vesicle size and number are regulated by a clathrin adaptor protein required for endocytosis. *Neuron* **21**, 1465-1475.

Zhang S. & Racaniello V. R. (1997). Persistent echovirus infection of mouse cells expressing the viral receptor VLA-2. *Virology* **235**, 293-301.

Zhou M. & Li F. (2008). Complete nucleotide sequence of a coxsackievirus B4 strain that establishes infection in ICR mice pancreas and induces glucose intolerance. *Anat Rec (Hoboken)* **291**, 601-609.

Zimmermann H., Eggers H. J., Zimmermann A., Kraus W., Nelsen-Salz B. (1995). Complete nucleotide sequence and biological properties of an infectious clone of prototype echovirus 9. *Virus Res* **39**, 311-319.

ORGINAL PUBLICATIONS