Integrin alpha V beta 3 as a target for treatment of rheumatoid arthritis and related rheumatic diseases

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A substantial and persuasive body of data now exists that supports the view that integrin alpha V beta 3 plays a critical part in activated macrophage dependent inflammation, osteoclast development, migration, and bone resorption, and inflammatory angiogenesis. All of these processes play an important part in the pathogenesis of rheumatoid arthritis (RA) and related arthropathies. Animal arthritis model data further support these concepts and also suggest that therapeutic antagonism of integrin alpha V beta 3 is worthy of further investigation in RA and related arthropathies. To this end, VITAXIN, also known as MEDI-522, has been developed. VITAXIN is a humanised monoclonal IgG1 antibody that specifically binds a conformational epitope formed by both the integrin alpha V and beta 3 subunits. It blocks the interaction of alpha V beta 3 with varius ligands such as osteopontin and vitronectin. Clinical trials with VITAXIN in patients with RA are in progress.

Although medical treatment for rheumatic diseases such as rheumatoid arthritis (RA) has improved dramatically in recent years (most notably with the development of anti-tumour necrosis factor α agents used in combination with methotrexate), treatment for a substantial number of patients with RA remains suboptimal. Most patients fail to completely respond to these new treatments, and for many other patients, the beneficial effects of these treatments diminish with time. Many of patients with RA still will ultimately require costly joint arthroplasties as an additional consideration, osteoclast mediated bone resorption, angiogenesis and pathological neovascularisation, and tumour metastasis. In general, integrins serve important cellular biosensing roles and convey signals from both outside the cell to inside (“outside-in” signalling) and, vice versa, from inside the cell to outside (“inside-out” signalling).

The integrin alpha V (CD51) subunit can form heterodimers with at least five distinct beta subunits: beta 1, beta 3 (CD61), beta 5, beta 6, and beta 8. The integrin beta 3 subunit, on the other hand, can associate with not only alpha V but also alpha IIb subunits (CD41). This molecular association information is important from the perspective of designing and assessing potential therapeutic monoclonal antibodies.

INTEGRIN ALPHA V BETAS: WHAT IS IT?
Integrins are heterodimeric transmembrane proteins formed by non-covalent association of alpha and beta subunits. Both subunits are type I membrane proteins with large extracellular ectodomains and short cytoplasmic tails. In mammals, the integrin family contains at least 18 alpha subunits that associate with at least nine beta subunits. The alpha and beta subunits assemble into at least 24 structurally distinct receptors. Ligands for these integrin receptors are diverse, but all use an acidic residue for recognition by the integrin. Specificity for an individual ligand is determined by additional contacts with the integrin. Ligand binding induces structural changes in the integrin and interactions with other molecules. In general, integrins serve important cellular biosensing roles and convey signals from both outside the cell to inside (“outside-in” signalling) and, vice versa, from inside the cell to outside (“inside-out” signalling).

INTEGRIN ALPHA V BETAS: WITH WHAT MOLECULES DOES IT ASSOCIATE?
Integrin alpha V beta 3 has distinct functional properties that are mediated through interactions with a variety of extracellular matrix (ECM) proteins in addition to vitronectin. These ECM proteins include osteopontin, fibronectin, fibrinogen, thrombospondin, proteolysed collagen, von Willebrand factor, and does not bind integrins alpha V beta 5 or alpha IIb beta 3.

The integrin alpha V beta 3, also known as the vitronectin receptor, consists of a 125 kDa alpha V subunit and a 105 kDa beta 3 subunit. It has been the focus of intensive research because of its major role in several distinct processes, particularly osteoclast mediated bone resorption, angiogenesis and pathological neovascularisation, and tumour metastasis. Evidence of the intense interest in this integrin is provided by the recent determination of the crystal structure of the extracellular segment of integrin alpha V beta 3. This work shows that the heterodimeric molecule can exist in either an extended or “flexed” conformation. The available data indicate that the “flexed” or “bent” conformation represents the inactive form. In addition, the crystal structure of alpha V beta 3 binding an Arg-Gly-Asp ligand has also been determined.

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Abbreviations: RA, rheumatoid arthritis; ECM, extracellular matrix
and others. The specific nature of the extracellular interactions of integrin alpha V beta 3 with ECM has additional functional effects on the expression of other cell surface receptors. For example, culturing endothelial cells on vitronectin increases the presence of VEGFR1, as well as VEGFR2, FGFR1, and FGFR2 on the endothelial cell surface. Each of these receptors plays an important part mediating the effects of angiogenic growth factors such as VEGF, FGFR1, and FGFR2. The increased expression of VEGFR1 and the other receptors is blocked with if anti-alpha V beta 3 and alpha V beta 5 antibodies are added to the culture system. In contrast, plating endothelial cells on fibrin, instead of vitronectin, decreases the expression of these receptors. Thus, these integrins function to transmit signals into the cell, and the cell responds by changing its expression of surface receptors—that is, in this case, the expression of angiogenic growth factor receptors.

Integrin alpha V beta 3 not only modulates the levels of expression of selected cell surface molecules, it also physically associates with a number of important cell surface molecules including VEGFR2, IAP, MTI-MMP, MMP2, and CD47. The associations with these various cell surface molecules is modulated by alpha V beta 3 interactions with various ligands, and vice versa, implying that alpha V beta 3 plays a critical role in regulating the localisation or clustering of important cell surface molecules involved in cell adhesion, growth, survival, migration, and invasion through the ECM. In addition to the ECM and cell surface membrane molecular associations, integrin alpha V beta 3 also interacts with a number of intracellular signalling molecules. These include paxillin, focal adhesion kinase, caspase 8, and others. These interactions also play a part in regulating intracellular signalling, cell migration, cell proliferation, and cell survival.

**INTEGRIN ALPHA V BETA 3: WHERE IS IT FOUND? WHAT TISSUES? WHAT CELLS?**

Alpha V beta 3 is expressed at low levels in most normal tissues including intestinal, vascular, and smooth muscle cells, but, of particular interest, is that high level expression is limited to bone, the mid-menstrual cycle endometrium, placenta, inflammatory sites, and invasive tumours. The cell types that express high levels of this integrin include mature, bone resorbing osteoclasts and activated macrophages, a small fraction of neutrophils, angiogenic endothelial cells, and migrating smooth muscle cells. These cell types clearly reflect the involvement of integrin alpha V beta 3 in bone resorption, neovascularisation, and inflammation. As pathological osteoclast mediated bone resorption, macrophage dependent inflammation and neovascularisation are characteristic features of diseases such as RA and related arthropathies, these observations provide the first line of supportive evidence that alpha V beta 3 may represent a rationale therapeutic target for these diseases. Pathological bone resorption and neovascularisation are also characteristic features of many malignant tumours. Moreover, expression of integrin alpha V beta 3 is also characteristic of several invasive tumour types including melanoma, glioma, ovarian, and breast cancer. These observations suggest that pharmacological antagonism of the integrin alpha V beta 3 may represent, in addition to rheumatic diseases such as RA, a rational approach to treatment of cancer.

**INTEGRIN ALPHA V BETA 3: WHAT DOES IT DO? BIOLOGICAL FUNCTIONS?**

From the preceding discussion of the integrin alpha V beta 3, it should be clear that this integrin, through interactions with ECM, various intramembranous and intracellular molecules, modulates the growth, survival, motility, and differentiation of a limited group of cells. These cells include osteoclasts, activated macrophages, and angiogenic endothelial cells. Cellular functional activities are regulated by changes in the affinity and avidity of the integrin, through changes in its phosphorylation state, the nature of ECM ligands, and other factors.

The function of the alpha V and beta 3 subunits has also been assessed through studies of gene knockout mice. About 20% of alpha V integrin knockout mice survive to delivery. Placentas from these mice show defects, and the mice have abnormalities in central nervous system and gastrointestinal blood vessels. Cleft palate is also a frequent abnormality. The alpha V integrin subunit associates with beta 1, beta 3, beta 5, beta 6 and beta 8 integrin subunits. Beta 3 integrin knockout mice are fertile and survive. Their important problems are defective platelet aggregation, defective osteoclast resorptive capacity, and osteosclerosis. These also have to propensity to permit transplanted tumours to grow more rapidly. The report that transplanted tumours grow more rapidly in beta 3 knockout mice has been subject of extensive controversy because the conclusions contrast strikingly with studies with integrin antagonists on tumour growth. In other words, conclusions from gene knockout and studies with integrin antagonists on tumour growth have not been consistent without more in depth analysis.

The functional role of integrin alpha V beta 3 has been most extensively studied in the context of osteoclastogenesis and bone resorption, macrophage migration and activation, and angiogenesis. The integrin alpha V beta 3 is highly expressed on activated macrophages and osteoclasts. These cell types are found in abundance at sites of bone destruction in RA patients—that is, activated macrophages are markedly increased in both subchondral bone and inflamed synovial tissues, and osteoclasts are markedly increased in subchondral bone at sites of bone erosion and resorption. In patients with RA, mature osteoclasts are highly associated with both periarticular and systemic bone loss.

Osteoclasts are highly specialised and differentiated multinucleated cells related to macrophages. Under normal physiological conditions, mature osteoclasts expressing alpha V beta 3 are continuously generated from immature bone marrow mononuclear precursor cells. Stimulatory factors such as macrophage colony stimulating factor (MCSF) and receptor activator of NF-κB (RANK) ligand (RANKL) largely determine this differentiation process. During inflammatory diseases such as RA, tumour necrosis factor α (TNFα) and interleukin 1 (IL1) also significantly amplify osteoclastogenesis and generation of activated macrophages. Studies of macrophages and osteoclasts have shown that blocking alpha V beta 3 inhibits adhesion, migration, and, for osteoclasts, bone resorption. Resorbing osteoclasts develop a specialised membrane domain termed the tight sealing zone or clear zone. Alpha V beta 3 and one of its ligands, osteopontin, are enriched in the clear zone of the resorbing osteoclast. The clear zone is believed to mediate attachment to the bone matrix and the formation of acidic resorption lacunae that are required for bone resorption. Other data suggest that alpha V beta 3 is involved in activated osteoclast and osteoprogenitor cell formation. Podosomes are involved in translocating signals that regulate cell attachment, survival, and function internally into the macrophage and osteoclast. Disrupting alpha V beta 3 signalling molecules such as gelsolin impairs podosome formation, cell movements and bone resorption. As osteoclast mediated bone resorption and macrophage dependent inflammation are such a central pathogenic feature of RA, these data provide a strong support for the concept that therapeutic inhibition of alpha V beta 3 is sensible.

Some of highest relative expression levels of alpha V beta 3 are observed on growth factor/cytokine activated endothelial cells, especially blood vessels in granulating wounds or tumours. It is also intensely expressed in inflamed synovial tissues of patients with RA and rabbits with experimental
patients may have therapeutic benefits.

**BLOCKADE OF ALPHA V BETA 3 IN PRECLINICAL RA MODELS**

A persuasive body of evidence indicates that alpha V beta 3 plays an important part in mediating the migration, differentiation, proliferation, and survival of a limited group of cells that express this integrin. It is particularly noteworthy that the cells that express the highest levels of alpha V beta 3 include activated macrophages, which are involved in producing proinflammatory cytokines, osteoclasts, which mediate inflammatory osteolysis, and endothelial cells, which are involved in pathological neovascularization. Macrophage differentiation, migration, and inflammatory osteolysis, and endothelial cell neovascularization are clearly involved in the pathobiology of RA. These observations have stimulated the notion that blocking the function of integrin alpha V beta 3 may have therapeutic benefit in RA, and this hypothesis has been explored to a limited extent in preclinical animal models of inflammatory arthritis.

Intraarticular administration of a cyclic peptide alpha V beta 3 antagonist to rabbits with antigen induced arthritis, a model with features that resemble RA, inhibits synovial angiogenesis, inflammatory cell infiltration, and bone and cartilage destruction. The antagonist treatment also has a disease inhibitory effect when given to animals with chronic disease. In addition, SB273005, a non-peptide antagonist of alpha V beta 3, was reported to significantly reduce symptoms and signs of adjuvant arthritis in the LEW rat. This arthritis model has a very a good record of predicting therapeutic efficacy in patients with RA. SB273005 is also a well documented inhibitor of endothelial cell migration in vitro and a potent inhibitor in several animal models of bone resorption in vivo. Oral dosing with SB273005, started before disease onset or at the time of disease onset, significantly inhibits joint swelling and the destruction of both bone and cartilage (36%–52%, depending on schedule and dose).

More recently, the role of osteopontin has been examined in an experimental RA model. Osteopontin is an extracellular matrix protein containing Arg-Gly-Asp (RGD) sequence, which interacts with alpha V beta 3 integrins. Its expression in the joints of a murine RA model was associated with joint swelling, destruction of the surface structures of the joint based on scanning electron microscopy, and loss of toluidine blue positive proteoglycan content in the articular cartilage in wild type mice. In contrast, osteopontin deficiency prevented the mice from such surface destruction, loss of proteoglycan in the articular joint cartilage, and swelling of the joints. These preclinical results provide further support to the evolving view that alpha V beta 3 is an appropriate target for treatment of RA and related diseases.

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