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 role 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 various 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 over the years, the recent determination of the crystal structure of the extracellular domains of the integrin alpha V beta 3 complex supports the view that integrin alpha V beta 3 plays a critical role in activated macrophage dependent inflammation, osteoclast development, migration, and bone resorption, and inflammatory angiogenesis. 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 various ligands such as osteopontin and vitronectin. Clinical trials with Vitaxin in patients with RA are in progress.

Integrin alpha V beta 3: what is it?

Integrins are heterodimeric transmembrane proteins formed by non-covalent association of alpha and beta subunits. Both subunits are type 1 membrane proteins with large extracellular ectodomains and short cytoplasmic tails. Integrins are involved in a variety of cellular processes, including cell adhesion, migration, and signaling.

INTEGRIN ALPHA V BETA 3: 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, and collagen.

Abbreviations: RA, rheumatoid arthritis; ECM, extracellular matrix
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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, FGFRI, and FGF2 on the endothelial cell surface. Each of these receptors plays an important part mediating the effects of angiogenic growth factors such as VEGF, FGF1, and FGF2. The increased expression of VEGFR1 and the other receptors is blocked with anti-alpha V beta 3 and alpha V beta 5 antibodies are added to the culture system. In contrast, plating endothelial cells on fibronectin, 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 part 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 resoring 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 pathologic osteoclasts, 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 realistic 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.

**INTEGRAL VEIN 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 resorative 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.

A 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-kB (RANK) ligand (RANKL) largely determine this differentiation process. During inflammatory diseases such as RA, tumour necrosis factor alpha (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 osteoclasts. 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 osteoclasts and osteoblasts. The formation of podosomes is involved in transducing 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...
RA-like arthritis. Alpha V beta 3 ligands, such as osteopontin and fibrinogen, are also abundant in rheumatoid synovial tissues. The interaction of these ligands with alpha V beta 3 seems to play an important part in development of the hyperplastic, tumour-like, invasive synovitis that contributes to destruction of bone and cartilage in RA. Endothelial cells in rheumatoid synovium are subject to continuous production of angiogenic stimuli (TNFα, VEGF, FGF1, FGF2), resulting in the expression of alpha V beta 3 on sprouting endothelial cell buds and new blood vessel development. Alpha V beta 3 plays a very important part in this process by facilitating the attachment to and migration through the ECM by the newly developing blood vessels. These observations also support the view that inhibition of alpha V beta 3 in the synovium of RA 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 pro-inflammatory cytokines, osteoclasts, which mediate inflammatory osteolysis, and endothelial cells, which are involved in pathological neovascularisation. Macrophage derived osteoclast precursors, inflammatory osteolysis, and angiogenesis 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 arthritides, 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 arthritic joints 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 toulidine 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.

REFERENCES


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