Editorial



# Reducing affinity of αvβ8 interactions with latent TGFβ: dialling down fibrosis

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The widely distributed, pleiotropic cytokine TGFβ has a critical role in development, immune function and tissue homeostasis (1), and aberrant TGFβ signalling has been implicated in the pathogenesis of numerous diseases in various organs. Excessive TGFβ signalling has been implicated in a number of fibrotic conditions in the lung including pulmonary fibrosis (2), airway remodelling in asthma (3,4), acute lung injury (5) and chronic obstructive pulmonary disease (COPD) (4). Unfortunately global inhibition of TGFβ leads to severe toxicity (6,7) and therefore there is considerable interest in strategies that can inhibit TGFβ signalling in a cell and tissue specific manner.

Regulation of TGFβ functions occurs primarily via its activation. TGFβ is released from cells non-covalently associated with its pro-peptide, also known as the latency associated peptide (LAP), creating a latent TGFβ complex which is sequestered in the extracellular matrix (ECM) through binding to ECM glycoproteins. Over the past 25-30 years multiple mechanisms of TGFβ activation have been described including both physical (such as extremes of heat or pH) and biological mechanisms (8). Several proteases have been shown to activate TGFβ via proteolytic cleavage of the LAP (8,9). Furthermore, the latent TGFβ complex can be activated by interactions with other proteins such as thrombonspondin-1 or several integrins, which induce a conformational change within the LAP resulting in the release of active TGFβ (8).

Integrins are cell surface heterodimeric receptors, composed of α and β subunits, that are responsible for cell-cell and cell-matrix interactions. Several integrins are able to bind to the latent TGFβ complex through RGD binding motifs in the extracellular domains. This interaction allows the integrin to act as a direct link between a cell and the latent TGFβ complex in the ECM, and enables the integrin to activate the latent TGFβ complex. Most TGFβ activating integrins (αvβ6, αvβ3, αvβ5 and αvβ1) activate TGFβ through mechanotransduction of intracellular force to the latent TGFβ complex, which creates a force-dependent conformational change in the latent complex resulting in the release of active TGFβ from the complex (10). However, αvβ8 integrins have a unique mechanism of TGFβ activation that involves recruitment of matrix metalloproteinase-14 (MMP-14) and proteolytic cleavage of the latent complex to release active TGFβ molecules (11).

In 2014, Minagawa and colleagues engineered an antibody directed against the αvβ8 integrin and demonstrated that it blocks αvβ8-mediated TGFβ activation with very high specificity and low off-target effects (4). Not only did they show that inhibiting this integrin protected mice from airway remodelling in response to adenoviral over-expression of IL-1 and combined cigarette smoke and Poly IC exposure, but they also addressed some central questions relating to αvβ8-mediated TGFβ activation. They show that the αvβ8 integrin is in a high affinity state constitutively bound to latent TGFβ. Furthermore, they demonstrate that the B5 antibody not only inhibits TGFβ activation through allosteric inhibition of binding to LAP, but promotes a low affinity state which does not alter cell adhesion, or inhibit binding assays for the αvβ6 integrin. These are fascinating studies that give real insights into αvβ8-mediated TGFβ activation and provide tools for dissecting the role of αvβ8 integrins in a number of conditions characterised by excess TGFβ activation which has not previously been possible.

Increased activation of TGFβ by the epithelial restricted integrin αvβ6 is a key driver of parenchymal fibrosis in the lung, liver and kidney fibrosis (2,12), however, loss of αvβ6-

mediated TGFβ activation promotes lung tissue destruction and emphysema (8). Force mediated activation of TGFβ by the αvβ5 integrin activation has been implicated in dermal and lung fibrosis (13-15), and αvβ1 integrins play a role in pericyte driven fibrosis in the lung, liver and kidney (16). The role that αvβ8 mediated TGFβ activation plays in parenchymal fibrosis remains unclear, especially where driven by epithelial TGFβ activation. It seems unlikely that there is a significant role of αvβ8 in epithelial TGFβ activation because the B5 antibody was reported not to affect alveolar size in the cigarette smoke model of COPD (4), although the duration of exposure may not have been sufficient to rule out an effect and further studies will be required to determine whether there are overlapping effects between αvβ8 and force mediated TGFβ activation.

Similarly integrin mediated TGFβ activation has profound effects on the immune response (17). Loss of αvβ8 integrins on dendritic cells promotes autoimmune colitis but protects against Th17 mediated encephalitis and parasitic infections (17-19). Therefore systemic administration of B5 antibodies will need to be carefully evaluated in a number of models before it can be developed as a therapy for airways disease, and it is possible that formulation of the antibody for aerosolised use will be a favourable option. Furthermore, influenza infection of epithelial cells results in αvβ6-mediated TGFβ activation, and inhibition of the αvβ6 integrin can prevent influenza induced collagen deposition within the lungs (20). Similarly poly IC enhanced, smoke induced, airway remodelling was inhibited by B5 administration (4) and it is thus possible that there are synergistic functions of these two integrins in response to viral infection. However, systemic inhibition both the αvβ6 and αvβ8 integrins recapitulate the highly proinflammatory phenotype of TGFβ1 and TGFβ3 null mice (7) highlighting the importance of understanding any overlapping, or cell specific activation, of TGFβ by these integrins in the lung.

What remains unclear, despite these elegant studies, is how B5 antibody binding to the αvβ8 integrin interacts with MMP14 and TGFβ to inhibit activation. It would seem likely that the low affinity binding induced by B5 prevents αvβ8 from tethering latent TGFβ sufficiently to facilitate proteolytic cleavage, thus it should prevent paracrine TGFβ signalling. The inability of force generated integrin mediated TGFβ activation to induce TGFβ signals in cells that are not in direct cell-cell contact is in contrast with αvβ8 integrin mediated TGFβ activation. However, the studies by Minagawa only describe the inhibition of TGFβ activity in co-culture assays with fibrosarcoma cells and thus could in theory be assessing force generated TGFβ activation. Whilst these cells do not express the αvβ6 integrin, nor does B5 interact with αvβ6, these cells do express the β1, and possibly β3 and β5 integrins. Whilst it seems unlikely that B5 will interact with these integrins, it would be interesting to know whether release of free TGFβ was also reduced and thus paracrine TGFβ signalling inhibited.

Targeting integrin-mediated TGFβ activation locally in diseased tissues represents a promising way of treating TGFβ-mediated pathologies such as organ fibrosis and tissue remodelling. The varying cellular and tissue specific distribution of integrin expression, and the fact that mechanisms of TGFβ activation differ between tissues provides the opportunity to reduce pathological TGFβ activation without affecting the normal homeostatic functions of TGFβ and further promoting disease. The studies described by Minagawa provide compelling evidence that reducing the affinity of integrin interactions with latent TGFβ can reduce TGFβ activation sufficiently to prevent tissue remodelling without completely abolishing TGFβ activation or other integrin functions such as cell adhesion, thereby potentially maintaining homeostatic functions of αvβ8 integrins. They also provide key insights into the mechanism of αvβ8 mediated TGFβ activation and generate tools which have the capacity to help further understand integrin mediated TGFβ activation in a range of fibrotic conditions.

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