

# ADAM Proteins as Therapeutic Targets in Loosening of Total Hip Replacement Implants?



ADAM (A Disintegrin And Metalloprotease) constitute a large and widely expressed family of type I transmembrane proteins with metalloprotease, disintegrin/cysteine-rich, epidermal growth factor-like, transmembrane and cytoplasmic domains<sup>1</sup>. About 40 ADAM have been identified in different species. ADAM can act as proteolytic enzymes — referred to as “sheddas” — in releasing membrane proteins from the cell surface or cleaving proteins of the extracellular matrix. Substrates of ADAM form a heterogeneous group of proteins including cell adhesion molecules, cytokines, and receptors<sup>2</sup>. There are 2 ways ADAM can modulate cell-cell signalling and cell adhesion: by the shedding of cell adhesion molecules or by direct interaction with cellular integrins via their disintegrin/cysteine-rich domain. Because intracellular domains of ADAM contain protein binding domains such as SH3 or SH2, they are capable of transducing cell signaling.

In many diseases, ADAM proteins are considered suitable targets for antiinflammatory therapies<sup>3</sup>. It is therefore essential to describe the ADAM proteins involved in discrete pathological states. In this issue of *The Journal*, Dr. Kontinen’s group investigate expression of an ADAM family member, ADAM12, in interface membranes around loosened total hip replacement implants<sup>4</sup>. Our editorial provides background on the possible roles of ADAM in this process.

Aseptic loosening is the major cause of failure in total hip replacement (THR). It is the result of wear-debris- or micromotion-associated inflammatory host response, which can lead to monocyte transmigration, activation of macrophages, formation of multinuclear cells, osteoclasts and foreign body giant cells, and, finally, to massive periprosthetic osteolysis.

Inflammation of the synovial-like interface membrane between bone and aseptically loosened prosthesis is considered to play the central role in aseptic loosening<sup>5</sup>. Typically,

synovial-like membranes consist of a combination of fibroblast-like cells, macrophages, and giant cells. Wear particles of the hip replacement implant or micromovement induce transmigration and activation of monocytes, leading to accumulation of macrophages in the synovial membrane. These macrophages are considered to have a dual role: (1) They induce host defense against particles via release of cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and regulate osteoclast formation, receptor activator of nuclear factor kappa B ligand (RANKL), and macrophage colony-stimulating factor (M-CSF)<sup>6,7</sup>. (2) They are precursor cells for osteoclasts responsible for bone resorption. Thus, aseptic loosening of THR implants depends on the regulated interplay of different cell types and their signaling through the extracellular matrix, and the 2 major events are inflammation and cell-cell fusion. For both events, a functional contribution of ADAM proteins has been described in pathologies of other tissues. Ma, *et al* analyzed ADAM12 expression in the synovial-like interface membrane around loosened THR implants. ADAM12 was mainly expressed in macrophage-like cells and multinuclear giant cells. Mononuclear ADAM12-expressing cells were detected close to multinuclear ADAM12-expressing cells. Ma, *et al* describe a strong correlation between ADAM12 expression and multinuclear cells; moreover, in longterm cell cultures, osteoclasts were also formed. Further, M-CSF and RANKL were detected in synovial membranes. Stimulation of monocytes with M-CSF and RANKL led to ADAM12 expression and to fusion in longterm stimulation. ADAM12, originally identified as a myoblast-expressed gene product, was expressed predominantly in skeletal muscle at embryonic and neonatal stages and in bone<sup>8</sup>. In C2 myoblast cells, ADAM12 was expressed in differentiation-induced myocytes and myotubes. In the same cell line, myotube formation was inhibited by suppression of ADAM12 expression, indicating that ADAM12 is impor-

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tant for myotube formation either by mediating cell-cell or cell-matrix interaction or by mediating fusion directly<sup>8</sup>. Recently, the interaction of the ADAM12 disintegrin/cysteine-rich domain with  $\alpha 9 \beta 1$  integrin was demonstrated to be instrumental for cell-cell fusions<sup>9</sup>. In addition, a secreted form of ADAM12 processes insulin-like growth factor binding proteins (IGFBP-3 and -5)<sup>10</sup>. ADAM12 has been detected in several cancer cell lines and tumors. In human giant cell tumors of bone, ADAM12 was increasingly expressed in multinuclear giant cells, which are likely osteoclastic and are seen in some mononuclear stromal cells. As increased ADAM12 expression correlated with the presence of multinuclear giant cells, it was proposed that the giant cells in these tumors form by fusion of ADAM12-expressing mononuclear stromal cells, supporting the idea that ADAM12 is involved in cell-cell fusion<sup>11</sup>. It is interesting to note that the prominent form of ADAM12 is a membrane-bound protein containing the disintegrin/cysteine-rich domain, from which the metalloprotease domain has been removed<sup>4</sup>. Thus potential therapies aimed to inhibit cell fusions must be directed against the disintegrin/cysteine-rich domain of ADAM12.

In addition to the work on ADAM12 published in this issue, an article from Dr. Konttinen's group was published in 2003<sup>12</sup> demonstrating a functional role for ADAM8 in osteolysis. ADAM8 was originally cloned from macrophages<sup>13</sup>. Later, ADAM8 was identified as active protease<sup>14,15</sup> with adhesive function of the disintegrin domain<sup>15</sup>. Analysis of ADAM8 knockout mice revealed no major abnormalities during bone development<sup>16</sup>. Under inflammatory conditions, e.g., stimulation with lipopolysaccharide, ADAM8 expression is upregulated in macrophages<sup>17</sup> and in activated glia cells, cells with immune function in the central nervous system, and a direct correlation between expression of ADAM8 and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) was reported<sup>18</sup>. Bearing in mind that TNF- $\alpha$  is the major element in periprosthetic osteolysis, as described by Schwarz, *et al*<sup>19</sup>, ADAM8 can be considered as part of the TNF- $\alpha$  signaling cascade, similar to the situation observed in the nervous system.

In osteoclasts, ADAM8 expression was increased compared to osteoclast precursor cells. The formation of osteoclasts was inhibited when ADAM8 expression was blocked at late stages of osteoclastogenesis, suggesting that ADAM8 expression is important for differentiation and fusion rather than for proliferation. Because a soluble form of ADAM8 containing the entire extracellular portion of protein induced osteoclastogenesis, it was proposed that ADAM8 did not mediate cell-cell fusion directly but via activation of the fusion process<sup>14</sup>. ADAM8 expression was also detected in the synovial-like interface membranes around loosened hip replacement implants, mainly in cell-rich regions with macrophage-like cell infiltration, and in small capillaries. Hardly any expression was detectable in fibrotic areas.

Based on stainings for ADAM8 and tartrate-resistant acid phosphatase (TRAP, a marker enzyme for osteoclast activity) the conclusion was made that ADAM8- and TRAP-expressing mononuclear precursor cells were in close spatial relationship with ADAM8/TRAP-expressing multinuclear giant cells, suggesting a role for ADAM8 in formation of foreign body cells and osteoclasts<sup>12</sup>. It is likely that the inflammatory state observed in loosening of THR implants induces ADAM8 and leads to osteoclastogenesis.

In summary, it is quite obvious that ADAM8 and ADAM12 act together in supporting processes associated with aseptic hip loosening. However, the regulation and the mode of action of both ADAM proteins are quite different: ADAM8 is a TNF- $\alpha$  target gene, whereas ADAM12 is induced by M-CSF and RANKL. Moreover, whereas ADAM8 function is related to inflammation and regulation of osteoclastogenesis, for which catalytic activity is required, ADAM12 is directly involved in cell-cell fusions resulting in multinuclear giant cell types. Interfering with the function of these ADAM in cell fusion and signaling would be a promising approach to suppress aseptic THR loosening and periprosthetic osteolysis. Apart from specific inhibition of ADAM8 proteolytic activity, this could be achieved by injection of specific antibodies or by application of small peptides interfering with binding of the ADAM12 disintegrin/cysteine-rich domain.

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