

Appendix II

Role of metallic particles and ions in ALTR/ARMD

It is important to briefly discuss here the dual role of metallic ions and particles in the onset and progression of the ALTR/ARMD reaction in the periprosthetic soft tissue and bone, which is also one of the most interesting topics of research regarding the occurrence of ALTR/ARMD associated with metallic wear debris generated at the bearing surface and/or modular junctions by various mechanisms. Recent, comprehensive reviews of the literature have provided valuable hypotheses of cell damage and toxicological aspects related to ALTR/ARMD and suggested lines of further research in this important area of interaction between metallic nanoparticulate wear debris and host [1-3]. The local adverse effects of metallic ions/particles can be summarized in three main categories: (a) macrophage toxicity with formation of oxidative stress and cell death; (b) lymphocyte-dominant reaction associated or not with transmural tissue necrosis and myotoxicity; (c) stimulation of macrophages leading to periprosthetic bone invasion with a variable degree of osteolysis possibly leading to aseptic loosening of implant components. The early literature on ALTR/ARMD focused on the MoM bearing surface of second generation HRA and THA and the differences in wear debris were mainly attributed to the bioreactivity of CoCr metallic nanoparticles and generation of free metallic ions interacting with synovial fluid and/or intracellular proteins forming organometallic complexes of undetermined and untested immunogenicity [4]. Around 2011, the attention shifted to the corrosion particles generated at the head/neck modular junction of MoM THA with MAS and the CoCr DMN/stem [5-8].

Although the role of size, composition, oxidative stability, and dose of the particles with the addition of more subtle elements such the composition of absorbed proteins has been considered fundamental in the biology of the cell reaction [9], the prevalent hypothesis of the onset of the lymphocytic dominant reaction is centered on the role of cobalt ions with quantitative correlation of the Co and Cr ion level in blood, synovial fluid, and periprosthetic soft tissue [10-12] with host response by the interplay of innate and adaptive immunity [13]. There is a general consensus in the literature that the presence of Co rich particles and/or Co ions are important for the development some aspects of the ALTR/ARMD: (a) it is a relevant agent in the development of ALTR/ARMD [14] with the potential to activate TLR4 on local immune cells similar to the response to bacterial endotoxin [15], (b) it generates reactive oxidase species (ROS) with subsequent oxidative stress of the macrophages and their death [16] and possibly affecting T-cell activation as shown *in vitro* [17]. A recent study has shown generation of ROS after exposure to Co ions and not to metallic nanoparticles *in vitro* [18]: however, experimental conditions may not reflect the complexity of the factors *in vivo*, especially if different wear particles are generated at MoM bearing surface and metallic junctions and interact with the joint fluid before being phagocytized by macrophages.

The role of soluble ions has been favored over particulate cobalt-alloy implant debris for the release of crucial cytokines for ALTR/ARMD [19] in spite of several reports showing lack of correlation between pseudotumors and metallic ion concentrations or Co to Cr ratio [20-22]. This indicates the important role of the variability in the chemical speciation of the particles released from different arthroplasty implants. It needs to be noted that the predominantly lymphocytic type of reaction with transmural soft tissue necrosis was not described before the use of second-generation MoM HRA and THA implants and Non-MoM THA with CoCr DMN, even in cases of catastrophic accumulation of abrasion metallic wear debris and very high blood and synovial fluid concentration of Co and less of Cr ions with systemic toxicity. Therefore, it seems reasonable to assume that metallic debris generated by various corrosion

mechanisms is necessary for this type of ALTR/ARMD and could also play a role in the occurrence of macrophage-mediated osteolysis with aseptic loosening of the implant components.

More recently, cohorts of patients implanted with a model of non-MoM THA with CoCr DMN and TMZF stem have shown a high prevalence of a lymphocytic-dominant reaction with soft tissue necrosis and slightly increased levels of serum Co (mean 5.4 and 8.6 µg/L) and Cr ions (mean 2.1 and 1.8 µg/L) [23, 24] with a variable loss of material from the neck piece taper junction [25], also documented radiologically [26] and histologically [27, 28]. This implant generated corrosion metallic wear only at the neck/stem modular junction without any additional wear from the bearing surface [29]. Particle wear analysis has shown aggregates of nanosize corrosion particles and correlation between complexity of particle composition and degree of synovial macrophage and lymphocytic inflammation in MoM HRA, MoM LHTHA w/wo CoCr MAS, and non-MoM THA with CoCr DMN and TMZF stem [29]. These data point towards an unprecedented occurrence of particle-related toxicity and immunogenicity in the cohorts of patients implanted with this configuration, regardless of patients' sex and age. The importance of the metal particles over the ions is also corroborated, although to a lesser extent, by the toxicity/immunogenicity of the corrosion particle aggregates generated at the head/neck junction of MoM LHTHA with or without CoCrMAS and non-MoM THA with metallic heads and various metallic neck tapers [30], and total knee implants with modular femoral component [31, 32].

Serum protein adsorption can vary according to particle physical and chemical features [33] and studies are needed to elucidate how metallic wear particles/ions generated by various types of corrosion exhibit a higher degree of local toxicity and immunogenicity than conventional metallic particles. Studies on the protein corona coating the nanoparticles and nanoparticle aggregates could shed light on pathways for the different occurrence of the lymphocytic dominant ALTR/ARMD [34-38] as well as the characterization of wear particles and metalloproteins in joint synovial fluid by different methods [39, 40]. Different corrosion modes dictated by different metal alloy microstructure can be important in the different immunogenicity/toxicity of wear particles of similar size and metal composition [41]. This tribological analysis might also predict which type of wear particles might be released *in vivo* and possibly their effect with pre-marketing studies performed with the aid of bioreactors [42].

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