Introduction: Periprosthetic osteolysis frequently associated with aseptic loosening is the major complication following total joint replacements. Generation of wear debris from articulating prosthetic joint surfaces and supporting cement are thought to be responsible for inciting a chronic inflammatory response eventuating in osteoclastic bone resorption and mechanical failure of the implant. Although in vivo, in vitro and retrieval studies indicate increased expression of pro-inflammatory cytokines by macrophages and fibroblasts in response to wear debris, very little is known about the way wear debris can modulate cellular responses to these cytokines [1]. In a complex cytokine environment, such as found in the synovial capsule after total joint arthroplasty (TJA), a number of pro- and anti-inflammatory cytokines co-exist and control each other. Inhibition of one or some may be the reason for activation of others. The present study indicates that particles of polymethylmethacrylate (PMMA) can inhibit interleukin (IL)-6 mediated JAK/STAT signaling. It was further observed that reversion by SB was only partial when particle treatment was for 1 hr (compare lanes 5 to 8). This indicates existence of other inhibitory mechanisms independent of MAP kinases.

Methods: Human PBMCs, isolated from healthy volunteers, or purified monocytes were exposed to PMMA particles (cell:Particle ratio, 1: 30) for different time periods, cells extracted with lysis buffer followed by running of the extracts on Western blots. Blots were sequentially probed with phospho-p38 specific, phospho-ERKs specific and p38 specific antibodies, and bands were visualized by enhanced chemiluminescence [3]. In other experiments, cells were treated with either p38 specific inhibitor SB 202190, or MEK1 (a primary MAP kinase in the ERK cascade) inhibitor PD 98059 [4], followed by treatment with PMMA particles for 15 min or 1 hr prior to addition of IL-6 for 12 min. Cell extracts were made in extraction buffer as in the other experiment, and extracts were analyzed by photocromoband mobility shift assay (EMSA) to monitor STAT activity using STAT specific DNA probe, hSIE. Extracts used in EMSA were also run on Western blot and the filter was probed with anti-Stat3 antibody to show that difference in STAT activity was not due to unequal protein amount.

Results: Based on our previous finding [3], we tried to determine whether inhibition of STATs by PMMA particles also relied on activation of MAP kinases. As shown in Fig. 1, in PBMCs, PMMA particles activated ERKs and p38 group of MAP kinases but not Jun kinases (data not shown). Activation or phosphorylation (phosphorylation of MAP kinases is prerequisite for their activation) of both kinases could be detected within 5 min of PMMA treatment, was highest between 15-30 min, and by 1 hr it was close to background level. To further verify the roles of MAP kinases in STAT inhibition, and also to determine which of the MAP kinases is involved, we treated purified human monocytes with either PD 98059, or SB 202190, followed by PMMA and IL-6. As shown in Fig. 2, only compound SB 202190, specific inhibitor of p38 kinase, but not PD 98059 could reverse the effect of PMMA on IL-6 triggered STAT DNA binding (compare lanes 2 to 5). This suggested involvement of p38 kinase in PMMA mediated inhibition of JAK/STAT signaling. It was further observed that reversion by SB was complete when cells were treated with PMMA for 15 min, but only partial when particle treatment was for 1 hr (compare lanes 5 to 8). This indicates existence of other inhibitory mechanisms independent of MAP kinases.

Conclusion: There has been a tremendous burst of activity in recent years to understand the molecular mechanism of MAP kinase action in several diseases along with development of drugs to inhibit these enzymes. There are now several MAP kinase inhibitors on the market which hold promise for in vivo use [5, 6]. Our work suggests that several of these MAP kinase inhibitors may have therapeutic potential for treatment of wear debris-mediated osteolysis and early implant loosening.

References: