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LACK OF TLR4 ON BONE MARROW DERIVED CELLS AMELIORATES EXPERIMENTAL ARTHRITIS AND DECREASES CD4⁺ IL-17⁺ CELLS

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Background and objectives The IL-1 receptor antagonist knockout mice (IL-1Ra^{-/-}) spontaneously develop a T cell driven arthritis with profound IL-17 production. When cross bred with TLR4 knock out (TLR4^{-/-}) these animals showed reduced inflammation, joint destruction, and diminished IL-17 levels.¹ Here, the authors set out to determine the role TLR4 on bone marrow derived cells in experimental arthritis.

Materials and methods An age-matched, sex-mismatched reciprocal bone marrow transplantation was performed with TLR4^{-/-} and TLR4^{+/+} mice in the IL-1Ra^{-/-} background. Reconstitution was assessed by staining bone marrow cells for Y-chromosome and peritoneal macrophages were stimulated with lipopolysaccharide (LPS). Clinical manifestation of disease was monitored macroscopically (0–2 per joint). Joint pathology and inflammation were scored on Safranin-O and haematoxylin/eosin stained sections (0–5). T cell analysis was performed on cells from draining lymph nodes using flow cytometry.

Results Reconstitution of bone marrow was successful as determined by Y-chromosome staining. Peritoneal macrophages from animals engrafted with TLR4^{+/+} bone marrow showed normal IL-6 mRNA up regulation after LPS challenge, whereas IL-6 mRNA up regulation was significantly reduced by 90% in animals that received TLR4^{-/-} bone marrow. Arthritis incidence was not affected by TLR4 deficiency and ranged between 50 and 87% in all bone marrow chimeras. However, animals that lacked TLR4 on the engrafted bone marrow cells, radio-resistant cells, or both showed 38 to 44% reduced arthritis score compared to animals expressing TLR4 on all cells. This decrease also reflected in bone erosion and cartilage scores of the joints, which were diminished in animals partially or complete TLR4 deficient. Cell analysis of draining lymph

nodes showed reduced percentage of IL-17+ cells. Strikingly, no differences were found in Th17 (CD4+IL-17+) levels, but in the CD4-IL-17+ population. When TLR4 was lacking on bone marrow cells the percentage of CD4- IL-17+ cells was reduced by 50%. Further analysis in IL-1Ra-/- mice showed that the CD4-IL-17+ cells in draining lymph nodes consisted for 80% of $\gamma\delta$ T cells. Indicating these cells as main IL-17 producers in the IL-1Ra-/- arthritis model.

Conclusions The role of TLR4 on bone marrow cells in experimental arthritis is promoting IL-17 production by CD4- cells, most likely $\gamma\delta$ T cells, and thereby aggravating arthritis. TLR4 increases macrophage activation which leads to attraction of $\gamma\delta$ T cells to the inflammation in the joint and subsequent IL-17 production at the inflammation site aggravating joint pathology.

REFERENCE

1. **Abdollahi-Roodsaz S**, Joosten LA, Koenders MI, *et al*. Stimulation of TLR2 and TLR4 differentially skews the balance of T cells in a mouse model of arthritis. *J Clin Invest*. 2008;**118**:205–16.