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## Activation of invariant NK T (iNKT) cells are involved in *Staphylococcus aureus* knee joint clearance

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**Background:** Invariant natural killer T (iNKT) cells are a unique subset of T lymphocytes which express a T cell antigen receptor (TCR) with an invariant variable α-segment 14–joining α-segment 18 (Vα14-Jα18) in mice or Vα24-Jα18 in humans (Kronenberg, 2005). *Staphylococcus aureus* (SA) is one of the dominant pathogens that induce septic arthritis in immunocompromised hosts (Goldenberg, 1998). **Objective:** In the knee joint *i*NKT cells are localized in the extravascular tissue surrounding the blood vessels. Therefore, we have investigated the role of *i*NKT cells in the initial state of joint arthritis induced by SA systemic infection. **Methods:** *Cxcr6*$^{gfp/+}$ knock-in mice on the Balb/c background and CD1d-deficient (*Cd1d*$^{-/-}$, Balb/c background) were used since most of CXCR6-GFP$^{+}$ cells are *i*NKT cells (Geissmann *et al*., 2005). Mice were infected with 1-5 × $10^{7}$ colony forming units (CFUs, iv.) of SA (strain USA 300). Spinning-disk multichannel-fluorescence intravital microscopy of the knee joint microvasculature was done as previously described (Andruski *et al*., 2008) 1-5 days after saline or SA inoculation. The activity of different cell types was assessed simultaneously using fluorescence-labeled antibodies against target cells. Measurements of knee SA CFU were also assessed. All protocols used were in accordance with the guidelines drafted by the University of Calgary Animal Care. Data within multiple groups were compared using an analysis of variance (one-way ANOVA) including a Newman-Keuls *post hoc* test for multiple comparisons. Data were considered statistically significant when $p<0.05$. **Results:** SA systemic administration (1-5 x$10^{7}$ CFU) caused a time-dependent decrease in animal weight and knee SA CFUs although significant numbers of knee SA CFUs were also detected at day 5 (2.4±0.9 x $10^{4}$ per 0.1g of tissue, n=5). Conversely, these effects were accompanied by a time-dependent increase in the number and activated state of extravascular CXCR6$^{+}$ cells in the knee microcirculation which were maximal 5 days after SA inoculation (44.3±7.9 vs. 64.9±8.7 CXCR6$^{+}$ cells per field of view, n=5, $p<0.05$). Administration of SA to mice deficient in iNKT cells (*Cd1d*$^{-/-}$) resulted in increased mortality and further reduction in animal weight. Interestingly, the number of knee SA CFUs was higher than in *Cxcr6*$^{gfp/+}$ animals (18.2±6.2 vs. 4.0±1.9. x $10^{4}$ per 0.1g of tissue, n=5, $p<0.05$). Administration of the CD1d ligand α-galactoceramide (2 µg/animal, iv.), a specific activator of iNKT cells, while having no effect on animal weight significantly diminished the number of knee SA CFUs compared with those detected in control mice (0.2±0.1 vs. 1.8±0.4 x $10^{4}$ per 0.1g of tissue, n=5, $p<0.05$). Notably, a greater increase in the number and activation state of knee iNKT than in *Cxcr6*$^{gfp/+}$ mice was observed (81.3±11.1 vs. 48.9±6.2 CXCR6$^{+}$ cells per field of view, n=5, $p<0.05$).

**Conclusions:** These results suggest that iNKT cells seem to protect against SA-induced joint arthritis probably increasing bacterial clearance.

**References:**

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