Supplemental Materials

Supplemental Methods

Reagents

All stocks were tested for endotoxin contamination using the Limulus Amebocyte Assay (Lonza) prior to use. The following anti-mouse antibodies were purchased from eBioscience: APC-labeled anti-Ly6G (Gr1) and anti-Siglec F (E50-2440); eFluor 450 labeled anti-F4/80 (BM8), anti-B220 (RA3-GB2) and anti-IA_b (AFG-120.1); AlexaFluor700-labeled anti-CD4 (GK1.5) and anti-CD11c (N418); PE and FITC-labeled anti-CD80 (16-10A1), PerCP Cy5.5-labeled anti-CD11b (M1/70) and CD44 (IM7), PE-Cy7-labeled anti-Ly6C (HK1.4) and anti-CD8 (536.7), Biotinylated and FITC labeled CD86 (GL1), FITC- and APC-labeled CD103 (2E7) and FcBlock anti-CD16/CD32 (93). The following anti-human antibodies were purchased from eBioscience: PE anti-CD16 (B73.1) and APC anti-CD66 (CD66a.B1.1). Beryllium ions in physiological solutions results primarily in the formation of $Be(OH)_2$ and thus this form of was used for mouse exposures[1]. $Be(OH)_2$ was diluted in either sterile saline or PBS prior to instillation. There was a slight difference in the pH of sterile saline (pH 6.5) and $Be(OH)_2$ diluted in saline (pH 7.0). However, we observed similar effects on IL-1α regardless of whether saline or PBS were used (Fig 1A, 2A). $Be(OH)_2$ particles were heterogeneous in size, ranging from 0.2-2.0 microns.

Isolation of human neutrophils

PBMC were separated from heparinized blood by Ficoll-Hypaque centrifugation. Pellets containing granulocytes and erythrocytes were subjected to sedimentation in a of 3% dextran (w/v) in sterile saline for 20 minutes at room temperature. The supernatant was removed and sedimented granulocytes were subjected to RBC lysis using a solution of ammonium chloride for 5 min. Granulocytes were resuspended in PBS for analysis.

Generation of LDLN single cell suspensions for analysis of DCs

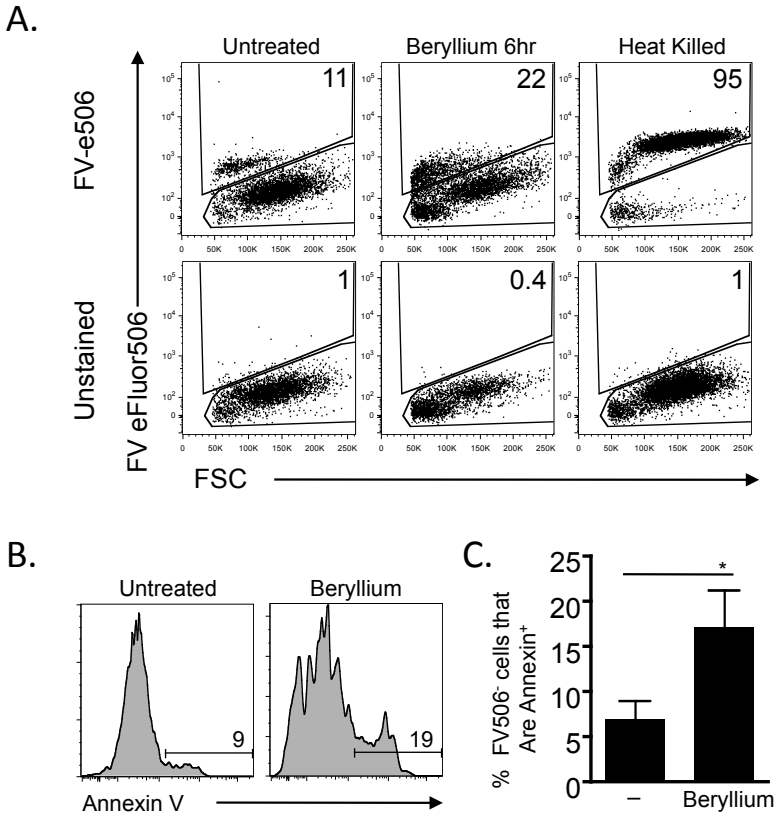
LDLNs were harvested into cRPMI on ice and immediately teased apart with 18 gauge needles. LNs were incubated with 1mg/ml Collagenase D and 100 μg/ml DNase I (both from Roche) in cRPMI for 30 minutes at 37° C. An equal volume of 100mM EDTA in PBS (pH 7.4) was added to the wells to stop the digestion and cells were washed through a 100 μm cell strainer, followed by a second wash of the wells with cRPMI media containing 5mM EDTA**.**

Generation of single cell suspensions from lung tissue Lungs were flushed of contaminating blood by placing a incision in the left atrium of the heart and flushing 10ml PBS through the lungs by insertion of a syringe and 25 gauge needle into the left ventricle of the heart. Lungs were minced with scissors and digested in cRPMI containing 1 mg/ml collagenase (Sigma-Aldrich). After 30 minutes, collagenase-digested lungs were disrupted sequentially by repeated passage through 16G and 18G needles. Cells were resuspended in PBS, erythrocytes were lysed with ammonium chloride and lung cells were filtered through 70 micron filters.

Generation of single cell suspensions for analysis of CD4$^+$ T cells LDLNs were harvested in cRPMI media on ice and immediately teased apart with 18 gauge needles. 1ml cold PBS was added to the surface of 70 μM cell strainers, and LNs were pushed through with 5ml cold PBS.

Supplemental Figures

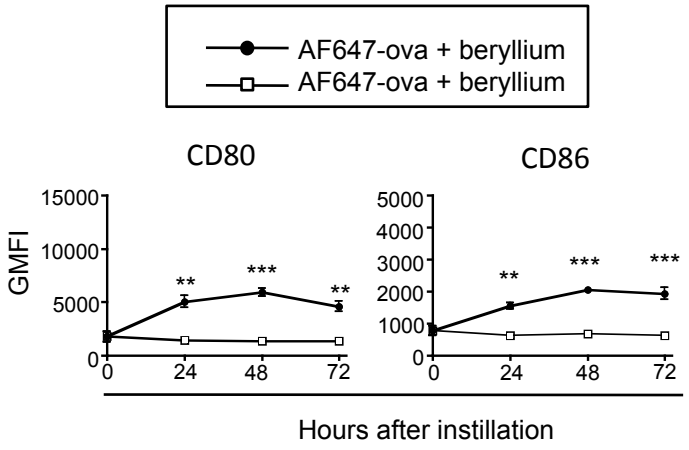
Figure S1.



Analysis of cellular death in BAL. A. Controls and gating for FV-e506$^+$ cells (late apoptotic/necrotic cells) in BAL harvested from untreated mice, mice treated i.t. 6hr previously with beryllium or heat killed BAL cells (incubated for 65C for 15 min) from

untreated mice. The top row shows cells stained with FV-e506 and the lower row shows unstained cells from the same mouse used. Percentages on graphs indicate the percent of cells that fall in the FV-e506$^+$ dead cell gate for each sample. **B.** FV-e506$^-$ cells were analyzed for early apoptosis by incubating with FITC-labeled Annexin V. Cells falling within the Annexin V$^+$ gate are shown for a representative example experiment of 2 separate experiments (n = 3 mice per experiment). **C.** Percentage (mean ± SEM) of FV506$^-$ BAL cells that were early apoptotic cells (Annexin V$^+$) in untreated mice and mice treated with $Be(OH_2)$ in PBS for 6hr are shown. The data are combined from 2 independent experiments (n=3 mice per group in each experiment).

Figure S2.



DCs in LDLNs of beryllium-exposed mice are globally activated. WT B6 mice were treated with AF647-ova (open squares) or AF647-ova + $Be(OH)_2$ (filled circles) as described in Figure 3. AF647-ova$^-$ cDCs were analyzed for expression (GMFI ± SEM) of CD80 and CD86. Data are representative of 3 separate experiments (n = 4 mice per group). A t-test was used to determine differences between groups at each indicated time

point. Significance levels are indicated by asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

References

1. Sutton, M. & Burastero, S.R. Beryllium chemical speciation in elemental human biological fluids. *Chem. Res. Toxicol.* **16**, 1145-1154 (2003).