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Supplementary Methods

Preparation of human HA fragments. Human HA fragments (M_w 200 kDa) were prepared as following. Serum was obtained from patients that met clinical criteria for the Adult Respiratory Distress Syndrome (~ 5-7 ml/patient) and was lyophilized and then fractionated on a Sepharose CL-4B column. Column fractions were analyzed for HA, protein and uronic acid content by HA ELISA as previously reported, absorbancy at 280 nm and carbozole reaction, respectively[1,2]. Low molecular weight (200 kDa) HA peaks were pooled, dialyzed and lyophilized. The low molecular weight HA was dissolved and precipitated in ethanol. This procedure was repeated 6 times. The HA was dialyzed, lyophilized, and then size fractioned on Sepharose CL-4B size exclusion chromatography as described previously[1]. The molecular weight of HA fragments used in the study had a peak ~ 200 kDa.

HA digestions and electrophoresis. HA fragments (M_w = 135 kDa, 1 mg/ml) were digested with hyaluronidase (from bovine testes, 100 Units/ml, Sigma) at 37 ^{0}C for overnight, Pronase (from Streptomyces griseus, 10 Units/ml, Calbiochem) at 55 ^{0}C for 1 hour, Deoxyribonuclease I (from bovine pancreas, 100 g/ml, Roche Applied Science) at 37 ^{0}C for 1 hour, and all samples were then boiled for 10 minutes. Digested HA samples along with known mw HA standards were electrophoresed on a 0.5% Agarose gel, stained, and photographed. The captured images were analyzed with ImageJ (National Institutes of Health) to determine HA size.

Exogenous KC treatment. Wild type and TLR2$^{-/-}$4$^{-/-}$ mice received bleomycin at 5 U/kg intratracheally, and one group of TLR2$^{-/-}$4$^{-/-}$ also received exogenous KC at a

dose of 1 g/mouse intramuscularly 1 hours post bleomycin and repeated daily. Five days after bleomycin induced lung injury, lungs were harvested, and total lung leukocytes and BAL leukocytes were analyzed.

Flow cytometric analysis. Mouse lungs were harvested after perfusion to remove blood and the lung cells were isolated by digestion in digestion buffer (150 U/ml Collagenase IV, 50 U/ml DNase I, 5% FCS in PBS) 37 °C for 30 min and minced against a filter. The lung cells or BAL cells were resuspended in PBS containing 1% BSA and 0.02% sodium azide. Non-specific binding was blocked by incubating with 25 g/ml Fc Block™ (BD Pharmingen) for 15 min at 4 °C. Samples were incubated with antibodies to neutrophil marker Gr-1, macrophage marker Mac-3, and lymphocyte marker CD3e (BD Pharmingen). Samples were washed twice with PBS and then fixed in 2% paraformaldehyde. Flow cytometry was performed after gating on the leukocyte population utilizing a FACSCalibur analytical flow cytometer (Becton-Dickinson) and analyzed using CellQuest Pro software.

Hyperoxia exposure. Adult 8-10-week-old $TLR2^{-/-}4^{-/-}$ and wild type mice were exposed continuously to 100% O_2 in a Plexiglas chamber as previously described[3,4]. Survival was monitored and evaluated.

References

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