Material and methods:

Lung resection specimens

Alveolar type II cells were extracted from patients undergoing lung cancer resection. We used cells from 14 donors for ATII cell extraction who had normal lung function (8M:6F, mean age 62.7 years). Resected lung specimens were immediately examined in the operating theatre by a member of the surgical team and a portion of specimen without macroscopic pathology and not required for a diagnostic purpose was passed to the research team. This sample was immediately immersed in sterile 0.9% saline in a sealed container and transported on ice to the laboratory for processing. The sample was measured and superficially washed with 0.9% saline immediately on arrival at the laboratory.

Primary human alveolar type II cells isolation and culture.

Primary human alveolar type II (AT II) cells were extracted according to methods described previously (15). Average yields of primary human alveolar type II cells were 30.2 million cells per resection with an average purity of 92%. Cells were tested for primary human alveolar type II (AT II) cell phenotype by alkaline phosphatase staining, lysotracker lamellar body staining and by PCR expression of surfactant protein C—a type II cell marker with negative expression of aquaporin V (a type I cell marker) (data not shown). 0.5 Million cells were seeded onto 24 well plates and

grown for 3 days in DCCM-1 (Biological Industries Ltd. Kibbutz Beit-Haemek, Israel) media supplemented with 10% fetal calf serum (FCS). Before stimulation cells were serum starved overnight (0.1% FCS) and stimulated in medium containing 0.1% FCS for 24 hours.

Primary human lung fibroblasts (HLF) were similarly cultured in dulbecco modified Eagle medium culture media (ECACC, Sigma, Poole,UK) supplemented with 10% FCS (sigma) at 37 °C and 5% CO_2. Cells were subcultured at 60-80% confluence using trypsin/EDTA. Cells were abtained from three separate donors, and all experiments were repeated in triplicate.

Stimuli and Inhibitors

AT II cells and fibroblasts were treated with LXA4 (Cayman Chemical Company, USA) at different concentrations. Inhibitors were used at the following concentrations according to manufacturers' instructions: LY294002, a PI3-kinase inhibitor (Calbiochem, Nottingham, UK) at 10 μM; and the nonselective FPR antagonist, Boc-2 (N-t-Boc-Phe-Leu-Phe-Leu-Phe; GenScript USA Inc), at 10μM. Inhibitors were added to cells 1hour prior to every treatment.

Bronchoalveolar Lavage Fluid Collection

BALF from ALI patients is known to stimulate alveolar epithelial cell repair in the scratch wound assay in an IL-1 dependet fashion (16). To

test whether LXA_4 could augment or synergise with this effect we used pooled BALF mmixed 50:50 with culture meda from patients with ALI as a positive control stimulus(16). We used BALF from patients enrolled into the BALTI-1 trial, demographics for whom have been published previously(17).

Real time PCR Assay

Real time PCR was performed using total RNA from primary human alveolar type II (AT II) cells and fibroblats (RNeasy Mini Kit; Qiagen, Hilden, Germany), the cDNA synthesis kit (MBI Fermentas, St. Leon-Rot, Germany), RNA (1ug) was DNase treated at room temperature and reverse transcribed using superscript RTase and random primers, according to the manufacturer's protocol. mRNA expression was analyzed using Taqman primer/probe (Applied Biosystems) and multiplexed with 18S to account for total loading. Relative mRNA amounts were calculated using *CT* method (18) $\Delta CT = Ct$ target-Ct GAPDH, $\Delta\Delta CT = Ct$ treatment–Ct calibrator, where calibrator was the no-treatment group. *Ct* was then converted to fold change using the formula $2^{-\Delta\Delta CT}$. Details of PCR primers are outlined in supplementary table 1 (Table S1):

Table S1

Details of primers used in Rel-time PCR

Gene name	Accession number	Forward primer	Reverse primer
Homo sapiens Vimentin	NM_003380	CTTCAGAGAGAGGAAGCCGA	ATTCCACTTTGCGTTCAAGG
Homo sapiens snail-homolog-2 (SLUG)	NM_003068	CAGACCCTGGTTGCTTCAA	TGACCTGTCTGCAAATGCTC
Homo sapiens collagen, type I, alpha 1	NM_000088	CTTTGCATTCATCTCTCAAACTTAGTTTT	CCCCGCATGGGTCTTCA
Homo sapiens collagen, type IV, alpha 1	NM_001845.4	CTAATCACAAACTGAATGACTTGACTTCA	AAATGGCCCGAATGTGCTTA
Homo sapiens actin, alpha 2, smooth muscle, aorta (ACTA2)	NM_001613	CCGACCGAATGCAGAAGGA	ACAGAGTATTTGCGCTCCGAA
Homo sapiens aquaporin 5	NM_001651.3	TaqMan® probe no. Hs00387048_m1	
Homo sapiens surfactant protein C	NM_001172357.1	TaqMan® probe no. Hs00161628_m1	
Homo sapiens advanced glycosylation end product-specific receptor (AGER)	NM_001136.4	TaqMan® probe no. Hs00542584_g1	
Homo sapiens S100 calcium binding protein A4 (S100A4)	NM_002961.2	TaqMan® probe no. Hs00243202_m1	

In Vitro Alveolar Epithelial Wound Repair Assay

Epithelial repair was determined using an in vitro epithelial wound repair assay as described before(19-20), Briefly, primary primary human alveolar type II (AT II) cells were grown to confluent monolayers before wounding with a 1-mL pipette tip. Cells were serum starved for 24 hours before wounding. After wounding, fresh basal media, bronchoalveolar lavage fluid and LXA4 at different concentrations was added to the

wounded monolayers. Digital images of the same point on the wound were taken at time 0 and at time 36 hours. Images were then analyzed using the Scion Image program by an operator blinded to the treatment conditions to avoid bias. To control for the inconsistencies in wound size, only monolayers in which the original wound areas varied by 10% of the mean were analyzed. Repair is expressed as the percentage of the original wound area covered by cells relative to control media. To allow for variability between cell types and batches, data are expressed as the mean (SE) percentage of control).

BRDU cell proliferation assay

4 $X10^5$cells/ml (AT II cells) or 1.5 $X10^5$cells/ml (HLF) were seeded into a 96 well culture dish. BrdU Label was added and cells were incubated with LXA4 or TGF-ß1 (R&D Sytems). After 24 hours culture, BrdU incorporation was assessed according to manufacturers' instructions (BRDU Cell Proliferation Assay, Promega ,UK).

Cell Titer Assay

After 24 hours of culture, viable cell count was assessed by adding 20 µL of Cell Titer 96 AQueous One Solution Cell Proliferation solution (Promega) to cells for 1.5 hours at 37°C and 5% CO_2. Data from proliferation bioassays comparing the Cell Titer 96 Aqueous Assay and

hydrogen-3–thymidine incorporation show similar results (21). Furthermore, in preliminary experiments, there was a linear relationship between cell titer readings and manually counted trypsinized primary AT II cells/ primary lung fibroblast cells over a range of cell counts (data not shown).

Flow Cytometry

Cells were left in serum-free media for 24 hours before exposure to 100 ng/ml Fas-ligand or Fas-ligand and TNF-α (R&D Sytems, Abingdon, UK). Apoptosis was determined by flow cytometry using the Annexin V and SyTOX antibody according to the manufacturer's recommendations (Molecular Probes, Eugene, OR) after 24 hours exposure.

Statistical Analysis

Data were normally distributed and analyzed by analysis of variance with Tukey's test for *post hoc* comparisons using Minitab 14.0 (Minitab, State College, PA). P value <0.05 was considered significant. Data are expressed as mean (SE).

Results

Figure S1. Effect of LXA_4 upon effects of soluble Fas ligand and TNF-alpha on proliferation and cell viability

A: sFasL and TNF-α inhibited cellular proliferation compared with control media–treated cells. This effect was attenuated by 100 nM LXA_4. Experiments were performed using cells from 3 donors.

B: Cellular viability of AT II cell was reduced 24 hours after treatment with 100 ng/mL sFasL or (and) 100 ng/mL TNF-α. Pre-treatment with LXA_4 at 100 nM significantly increases the viability of sFasL or (and) TNF-α treated cells after 24 hours.