**ONLINE SUPPLEMENTAL INFORMATION**

**METHODS**

**Celecoxib and Paracetamol Dosing Regime**

Mice were injected daily for 10 days intraperitoneally (i.p.) with either 10 mg/kg of celecoxib (Axorra, UK) or 125 mg/kg of paracetamol (Glaxo SmithKline, UK) in a final volume of 100 l, with the first dose administered 12 hours after the induction of influenza.

 The dose of celecoxib was selected based on previously published reports of therapeutic doses in mice.[25] In the case of paracetamol, the dose of 125mg/kg was selected as this dose exhibits similar pharmacokinetics to those observed in humans given therapeutic doses i.e. the drug reaches a peak in plasma between 30 minutes and 1 hour post-administration and exhibits a half-life of around 2 hours.[26] The therapeutic range for mice however, differs to that in humans. Whilst the mice received a dose of paracetamol which on weight basis alone is approximately double that given to humans (30mg/kg/day), this dose is at least three times lower than the mouse hepatotoxic dose (400mg/kg).[27] Indeed, no toxic effects were observed in our study as assessed by weight loss and serum ALT measurements.

**Quantification of pulmonary PGE2 during influenza infection**

Lungs were perfused from mice sacrificed at day 7 post infection, extracted and snap frozen in liquid nitrogen. Each lung was weighed and ground with liquid nitrogen in a pestle and mortar in PBS with butylated hydroxytoluene (BHT) (100 M) and diethylenetriamine pentaacetic acid (DTPA) (100 m). 10 ng of PGE2-d4 was added to each sample as internal standard. Lipids were extracted by adding a solvent mixture (1M acetic acid, isopropanol, hexane (2:20:30, v/v/v)) to the sample at a ratio of 2.5 ml solvent mixture/1 ml sample, vortexing, and then adding 2.5 ml hexane. After vortex and centrifugation, lipids were recovered in the upper hexane layer. The samples were then re-extracted by addition of an equal volume of hexane. The combined hexane layers were dried and analyzed for free or esterified HETEs using LC/MS/MS. For this, extracts were separated by reverse phase HPLC on a C18 Spherisorb ODS2, 5 m, 150 x 4.6 mm column (Waters Ltd, Herts UK) using a gradient of 50 – 90 % B over 10 min (A, water:acetonitrile:acetic acid, 75:25:0.1, B, methanol:acetonitrile:acetic acid, 60:40:0.1) with a flow rate of 1 ml.min-1. Products were quantitated by on an Applied Biosystems 4000 Q-Trap using parent to daughter transitions of *m/z* 351.2 to *m/z* 271 (PGE2, [M-H]-), and *m/z* 355.2 to 275.1 for PGE2-d4 with collision energies of -20 to -30 V. Products were identified and quantified using standard curves run in parallel under the same conditions.[28]

**Bronchoalevolar lavage (BAL)**

Mice were sacrificed and the lungs lavaged immediately with 1 ml of PBS/EDTA. Approximately 90 % of the total instilled volume was routinely recovered. BAL fluid was kept on ice and centrifuged at 3000 RPM for 10 minutes at 4°C. Cells were resuspended and used for cellular quantitation by flow cytometry.

**Lung cellular suspensions**

Following BAL extraction, lungs were perfused with 5 mls of PBS and the left-hand lobes extracted and stored in PBS on ice. Lungs were minced into small pieces and digested in 1mg/ml, 2ml final volume of collagenase D (Roche, Germany)for 45 minutes at 37°C with gentle agitation. Following digestion lungs were passed through a 70m cell strainer and centrifuged at 1500 RPM for 5 minutes. The pelleted cells were resuspended and used for cellular quantitation by flow cytometry.

**Flow cytometry**

Cells isolated from the lungs and BAL were stained with flurochrome-conjugated antibodies or MHC tetramers. Anti-NK1.1-FITC (BD, USA), anti-CD3-PerCP (BD, USA), anti-CD11c-PE-Cy7 (BD, USA), anti-GR-1-V450 (BD, USA), anti-CD11b-APC-Cy7 (BD, USA), anti-CD4-Pacific Blue (BD, USA), anti-CD8-PerCP-Cy5.5 (BD, USA), anti-CD8-APC-Cy7 (BD, USA), anti-IFN-PerCP-Cy5.5 (BD, USA), anti-7/4-PE (AbD Serotec, UK), anti-F4/80-APC (AbD Serotec, UK), and anti-TNF-FITC (eBioscience, USA) were used throughout these studies. The APC-labelled tetramer (Db-ASNENMETM) used in this study was generated in-house using previously described methods.[29] Cells stained with directly conjugated antibodies were incubated with 1-2 g/ml of antibody for 30 minutes at 4 °C prior to washing and resuspending in FACS buffer (PBS, 2 % FCS, 2 mM EDTA). Cells were fixed with FACS Fix buffer (PBS, 2 % FCS, 2 mM EDTA, 2 % Formalin) prior to analysis. In all cases the cells were analyzed by flow cytometry (Cyan ADP, Beckman Coulter, USA) and the data analyzed using Summit Software (DAKO, Colorado, USA).

For intracellular staining, single cell suspensions were stimulated by culture at 37 °C with PMA & ionomycin for 5 hours, GolgiPlug (BD, USA) was added for the last 4 hours. After incubation, cells were incubated according to supplier instructions with Live/Dead Fixable Dead Stain Kit Aqua (Invitrogen, UK) to enable detection of necrotic cells during analysis. Cells were washed and subsequently surface stained for CD4 & CD8 for 30 minutes at 4 °C, washed, fixed and permeabilised (Foxp3 staining kit, eBioscience, USA), blocked for 15 minutes with FcBlock (BD) and stained for TNF and IFN for 30 minutes at 4 °C.

***In vivo* lung function**

Airway function was measured in unrestrained, conscious mice by barometric plethysmography using a single chamber, whole body plethysmography system (Buxco Research Systems, Winchester, U.K.).[30] An increase in enhanced pause (Penh) was used as a measure of *in vivo* decreases in airways conductance and bronchoconstriction in response to inhaled methacholine 7 days after viral inoculation.[31] Baseline Penh was measured by placing the animals singly in the chamber for 1 minute before a 5 minute exposure to 10mg/ml methacholine aerosol delivered into the chamber by an Aerogen nebulizer (Buxco Research Systems). Peak changes in Penh were expressed as percentage of the baseline (mean ± standard error of the mean, SEM).

**Quantification of serum alanine aminotransferase (ALT)**

Serum was extracted from mice at day 7 post influenza infection. Serum ALT levels were determined by the Clinical Biochemistry Department at the University Hospital Wales, Cardiff, UK.

**In vitro virus quantitation assay**

The right-hand lung lobes were extracted following perfusion and placed immediately into 1ml of serum-free IMDM on ice. Lungs were homogenized and stored at -80°C. Lung viral load was determined using the previously described virus titration assay.[32] Briefly, serial two-fold dilutions of lung homogenates were incubated with MDCK cells with a methylcellulose overlay in a 24 well plate for 48 hours. Cells were fixed, permeabilised and stained with a monoclonal antibody specific for the PR8 haemagglutinin, for X31 an anti-influenza nucleoprotein antibody (AbD Serotec, UK) was used. Cells were incubated with a peroxidase-labelled secondary antibody (Bio-Rad, USA) and the plate was developed using AEC reagents (Sigma, UK). Each stained cell was counted and the total pfu/lung was calculated.

**Statistical Analysis**

Statistical analysis was performed using GraphPad Prism 3.0 software. A Kolomgorov-Smirnov test was performed upon each data set to determine if the data was normally distributed. The statistical differences indicated in Figure 1A–F and Figure 3 between influenza infected untreated mice and those treated either with celecoxib or paracetamol were calculated using one-way analysis of variance (ANOVA) followed by a Tukey-Kramer multiple comparison post-hoc test, used to compare multiple means. The statistical differences indicated in Figure 2C and D and Figure 6B, were determined using One-way-ANOVA with Tukey-Kramer post-hoc testing, this time comparing between uninfected naïve mice and those infected with influenza with or without treatment. The statistical differences in Figure 1G were determined by an unpaired T test, comparing between uninfected and influenza infected mice with or without treatment P values of < 0.05 were considered significant, with values of < 0.01 considered highly significant.