Transforming Growth Factor - β1 Suppresses Airway Hyperresponsiveness in Allergic Airway Disease.

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Online Data Supplement

Methods

Model of Allergic Airway Disease: Allergic airway inflammation was induced as previously reported (18). Briefly, mice were injected intraperitoneally (ip) with 40 μg of OVA in the adjuvant aluminum hydroxide (Alum) (Pierce Chemical, Rockford, IL) on days 1 and 14 to induce sensitization. Sham sensitized mice received Alum alone. Mice were then exposed to an aerosolized 1% OVA solution in sterile phosphate buffered saline (PBS) for 30 minutes on day 21 (1x), days 21 through 23 (3x), or days 21 through 26 (6x). Following OVA antigen challenge, lung tissues were harvested 2 days following the final OVA challenge. In TGF-β time-course studies mice were killed from 2 to 180 days following antigen challenge. TGF-β1 kinetic studies were performed by killing mice 15 minutes to 60 days following 1, 3, or 6 OVA challenges. Airway inflammation and TGF-β levels were assessed by bronchoalveolar lavage (BAL) with 1 ml of PBS.

Respiratory Mechanics: Airway resistance and tissue elastance were measured using the forced oscillation technique as previously described (19). Briefly, mice were anesthetized with 90 mg/kg of pentobarbital given ip. A tracheotomy was then performed using a modified 18 gauge IV adaptor and mice were attached to a computer controlled piston ventilator (Flexivent, SCIREQ Inc. Montreal, QE, Canada). Mice were ventilated with a tidal volume of 0.2 ml with a positive end expiratory pressure of 3 cm H_2O. Pressure was measured at the airway opening and volume was measured as the displacement of the piston of the ventilator. Multiple linear regression was used to fit measured pressure and volume in each individual mouse to the model of linear motion

of the lung (20, 21). Model fits that resulted in a coefficient of determination less than 0.85 were excluded.

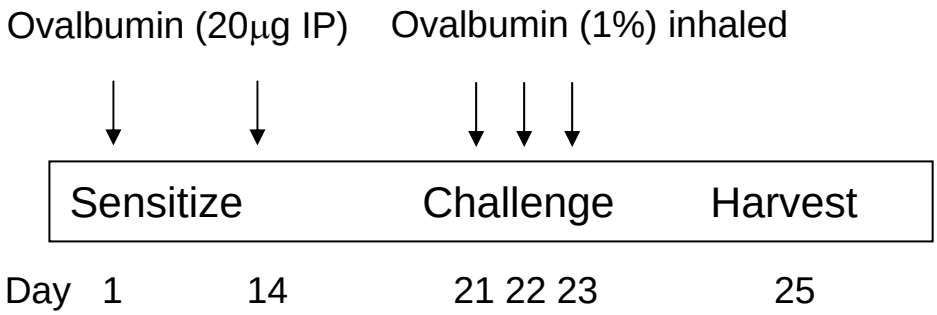
Determination of AHR: Dose-response curves to inhaled methacholine (Mch) were determined as follows. Mch was diluted with sterile PBS to concentrations of 0, 3.125, 12.5, 50 mg/ml and delivered sequentially via a nebulizer (Mystique, Airsep, Buffalo, NY). To expose the animal to aerosol, the input air line was diverted through the nebulizer, the tidal volume increased to 1.0 ml and the respiratory rate slowed to provide 20 large breaths of aerosol. Following each dose, the response was measured by applying a 2 second pertubation described above every 10 seconds for a total of 3 minutes.

Lung Histology and Immunohistochemistry: Lungs were inflated and fixed with 4% paraformaldehyde followed by paraffin imbedding. Blocks were cut into 5 μm sections and mounted onto slides. Airway inflammation was assessed by hematoxylin and eosin staining. TGF-β1 immunohistochemistry was performed on lung sections by a three-step indirect method. Antigen retrieval was done by incubation of slides for 20 minutes in 0.01 M sodium citrate pH 6.0 at 95ºC. Slides were then blocked with 2% normal goat serum for 30 min. Successive incubations in avidin for 20 min and biotin for 20 min were then performed to eliminate endogenous biotin activity. Sections were then incubated with polyclonal rabbit antibody against TGF-β1 (Santa Cruz Biotechnology, Santa Cruz, CA) (1:100 dilutions) overnight at 4˚C. Biotinylated anti-rabbit IgG was then applied for 30 minutes at room temperature, followed by addition of the avidin-biotin-complex-

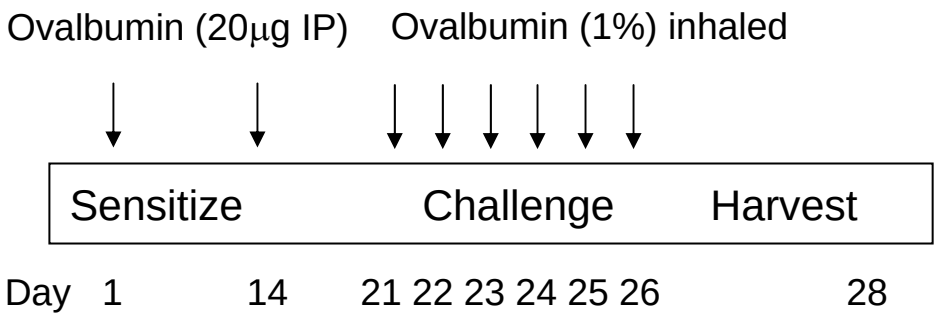
alkaline phosphatase (Vectastain ABC-AP, Vector Laboratories, Burlingame, CA) for another 30 minutes at room temperature. After rinsing the sections in PBS, the substrate, Vector® Red (Vector Laboratories), was added for 20 minutes. The Vector® Red reacts with the bound alkaline phosphatase, producing an intense red color. Phospho-Smad 2 staining was performed according to the antibody manufacturer's instructions with the substitution of AlexaFluor 568 (Molecular Probes, Eugene, OR) secondary antibody and nuclear Sytox Green (Molecular Probes) counterstaining. Images were generated by confocal microscopy.

Figure E1

3 Challenge Ova Model



6 Challenge Ova Model



TGF-β1 Antibody Ova Model