

SUPPLEMENTARY MATERIALS & METHODS

Generation of *L2-IL5* transgenic mice

The expression of a 5.5-kb IL-5 cDNA/genomic fusion gene [1] was driven using a promoter derived from the Epstein Barr Virus ED-L2 (EBV-ED-L2) gene [2, 3]. This transgenic construct was created in a shuttle-vector engineered from the plasmid pNNO3 (a kind gift of R. Tizzard, Biogen, Cambridge, MA), excised from plasmid sequences by NotI digestion, and injected into embryos derived from a cross of C57BL/6J mice. Transgenic positive founder animals were identified by PCR of DNA derived from tail snips using primers derived both the EBV-ED-L2 promoter (P1: CCTGTCTCCCACCCAGTAACTC) and the IL-5 cDNA-genomic fusion gene (P2: AGTCAGAACAACACTCAAGTGCAGAAGC). Subsequent generations of transgenic animals were the result of continued crosses onto the inbred strain C57BL/6J. Animals reported here were maintained in micro-isolator cages housed in a specific pathogen-free (SPF) animal facility, initially at Mayo Clinic Arizona and subsequently at the University of Colorado Denver. Age matched Wild Type C57BL/6J littermate mice were used as controls for all experiments. Mouse studies were approved by the University of Colorado Denver Institutional Animal Care and Use Committee.

Induction of experimental oesophageal eosinophilia in *L2-IL5* mice by sensitization and topical challenge with oxazolone

A mouse model of oesophageal inflammation was established using a 4-Ethoxymethylene-2-phenyl-2-oxazolin-5-one (oxazolone or OXA) (Sigma, St Louis, MO) contact hypersensitivity protocol (Figure 1). On day 0 of the oxazolone hypersensitivity protocol, a 2cm x 2cm area of abdominal skin of anaesthetised mice was shaved and oxazolone was applied to the skin surface (150µl of a 3%(w/v) solution of OXA in 4:1 acetone-olive oil vehicle) to initiate the sensitization phase of the protocol.[4] OXA sensitised mice were administered an oesophageal gavage of 1%(w/v) OXA in 30% ethanol/olive oil vehicle into the proximal oesophagus was performed on

protocol day 5, 8, and 12 (challenge phase of the protocol). Vehicle control animals (either Wild Type or L2-IL5 mice) were sensitised as noted above and challenged with 4:1 acetone-olive oil vehicle alone. All mice were assessed 24 hours following the last OXA challenge (protocol day 13).

Dexamethasone treatment

Dexamethasone in some studies was administered to individual mice on protocol day 5, 8, 10, and 12 by intraperitoneal (*i.p.*) injection of 200µg dexamethasone (Vedco, St. Joseph, MO) suspended in 200µl of saline (Figure 1). Control mice received 200µl of saline alone.

Tissue collection and histologic analysis

Tissues were fixed with 10% neutral buffered formalin and processed in preparation of being paraffin embedded [5]. Serial sections from these paraffin tissue blocks were cut (5µm) and were either stained with hematoxylin and eosin (H&E) or subjected to immunohistochemistry using either an anti-mouse eosinophil major basic protein-specific rat monoclonal antibody [6], or the Ki67 monoclonal antibody to assess cell proliferation [7]. Oesophageal inflammation at the peak site was measured from the H&E stained sections of each tissue based on a previously published method modified for use in mice by a board certified pathologist (KC) without knowledge of experimental conditions (Supplementary Table 1) [8]. Briefly, sections of each oesophagus were assessed for the presence of eosinophilic abscesses, presence of lymphoplasmacytic infiltrates, keratosis, dilated inter-cellular spaces and extent of basal zone hyperplasia. Scores were summed with a maximum possible score of 7.

Immunohistochemical/Immunofluorescence assessments of eosinophil inflammation and cell hyperplasia

Eosinophils were localised in the oesophageal tissue sections by immunohistochemistry using a rat anti-mouse eosinophil major basic protein-1 monoclonal antibody (Clone MT-14.7) as previously described [5]. Similarly, Ki67 immunostaining was used to identify proliferating cells as performed previously using a monoclonal rat anti-mouse Ki67 antibody (Clone TEC-3; Dako, Carpinteria, CA) [7]. MBP-1 immunohistochemical positive cells were identified through visualization of a permanent red chromotrope and Ki67 immunohistochemical positive cells were identified through visualization of a DAB chromotrope (Dako, Carpinteria, CA) with the slides counterstained with Methyl Green (Sigma, St Louis, MO). Negative control sections were performed by replacing the primary antibody in each case with a rat IgG isotype control antibody (Vector Labs, Burlingame, CA). Quantification of either MBP-1 (i.e., eosinophils) or Ki67 immunopositive cells are presented as numerical averages of nine non-overlapping high-power fields (0.26mm²) per oesophagus (3-distal, 3-mid and 3-proximal). Cell numbers are presented as a mean \pm SEM.

In those cases where infiltrating eosinophils were detected by immunofluorescence, tissue sections were incubated with Alexa Fluor 488 conjugated anti-rat antibody (Invitrogen, Grand Island, NY) and subsequently with PE-conjugated anti-SiglecF antibody (E50-2440; BD-Biosciences, San Jose, CA). Slides subjected to immunofluorescence staining were counterstained with the DNA-specific (i.e., nuclear) dye 4',6-Diamidino-2-Phenylindole, Dihydrochloride (DAPI) (Invitrogen, Grand Island, NY) prior to microscopy.

Cell Isolation and Quantification

Leukocytes from various tissue sources were isolated as previously described.[5, 7, 9] In the case of circulating leukocytes [9], whole blood was collected in BD-Biosciences EDTA treated

microfuge tubes (BD Biosciences, San Jose, CA). Splenocytes or bone marrow cells isolated from femurs and tibias of the hind limbs of subject mice were recovered using sterile PBS. Spleens were first minced into small pieces and dissociated into single cells. Bone marrow was dissociated into single cells by repeated pipetting of the recovered marrow sample. In both cases, clumps of cells and other debris were removed by filtration thru a 70 μ m cell strainer. Oesophagi were resected, cut longitudinally to allow the entire luminal surface to be exposed, washed in PBS and then the tissue digested with collagenase (Sigma, St Louis, MO) as previously described for other intestinal tissues [5]. Spleen, blood, bone marrow, and oesophageal cell preparations were removed of red blood cell contamination with Red Blood Cell lysis buffer (Sigma, St Louis, MO) as per the manufacturer's instructions and the recovered white blood cells were counted following dilution in 1X PBS and trypan blue dead cell exclusion using a hemocytometry.

Flow cytometric and fluorescence activated cell-sorting (FACS) analysis

Single-cell suspensions were stained for 25 minutes on ice with cell type-specific antibodies after blockade of Fc receptors using 1 μ g/ μ l of Fc blocker (CD16/32; BD Biosciences, San Jose, CA). Antibodies used for the staining of specific cell surface markers include CD103 (2E7), IL-5R α (T21), CCR3 (557974), SiglecF (E50-2440) all (BD-Biosciences, San Jose, CA); MHC-II (M5/114.15.2; Biolegend, San Diego, CA) and CD4 (GK1.5), CD8 (Ly-2), CD11b (M1/70), CD11c (N418), CD34 (RAM34), CD45 (30-f11), F4/80 (BM8), Ly6G (1A8), SCA-1 (D7) all (eBiosciences, San Diego, CA). Viable cells were selected for analysis with the use of Live/Dead AquaVi staining (Invitrogen, Grand Island, NY). Fluorescence analysis was performed using a BD FACSCanto™ II (BD Biosciences, San Jose, CA). Fluorescence activated cell sorting (FACS) was performed using a BD FACSAria (BD Biosciences, San Jose, CA). Data files were further analyzed using FLOWJo software (Tree Star Inc, Ashland, OR).

Sorted cells were cytospun onto charged glass slides and stained with DiffQuick (commercial Romanowsky stain) (Fisher, Pittsburgh, PA) for differential analysis.

RNA isolation and real-time reverse-transcription polymerase chain reaction (rtRT-PCR)

Tissue total RNA was prepared using RNeasy Mini Kits (Qiagen, Valencia, CA) and a hand-held laboratory homogeniser (PRO Scientific, Oxford, CT) as previously described [5]. First strand cDNA synthesis was performed from 500ng of total RNA using the High Capacity cDNA archive kit (Applied Biosystems, Foster City, CA) for all gene transcripts assessed in this study. Transcript expression was assessed using Taqman Gene Expression Assays Taqman probes (Applied Biosystems, Foster City, CA). rtRT-PCR reactions were performed with ABsolute™ Blue QPCR ROX Mix (Thermo Scientific, Surrey, UK), cDNA and Taqman assay mixtures. Thermocycling and subsequent analysis was performed with ABI-7300 System and software. Data was normalised to 18S expression and calculated as RQ (Relative Quantity; $2^{-\Delta\Delta C_t}$, where C_t is cycle threshold) for each sample. RQ values are presented as fold change in mRNA expression relative to Wild Type age-matched littermate controls unless otherwise stated.

Cytokine assessments of oesophageal tissue and blood serum

Oesophagi were resected, cut longitudinally to expose the luminal surface, and then washed in PBS. These oesophageal *ex plants* were cultured in complete RPMI medium 1640 (5% FBS, 2mM L-glutamine, 100IU penicillin and 100µg/mL streptomycin). Specifically, 2cm sections were cultured at 37°C for 24 hours and supernatants were harvested by centrifugation at 18,000g for 10 minutes at 4°C. Serum was isolated from whole blood as previously described [5]. Culture supernatants from *ex plant* cultures or blood serum samples were stored at -80°C prior to use. Assessments of cytokine/chemokine levels were performed by ELISA (as per the

manufacturer's instructions) - IL-5 (BD Biosciences, San Diego, CA); eotaxin-1 and eotaxin-2 (R&D Systems, Minneapolis, MN).

Statistical analysis

Statistical analyses of data outcomes were performed using a 2-tailed Student's *t*-test. Data are expressed as means \pm SEM. A *P*-value of ≤ 0.05 was considered statistical significance although in some cases higher levels of significance are noted and described in the figure legends where applicable.

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