METHODS

Preparation of knob protein

Plasmid constructs (pRSET A; Life Technologies) that produce knob protein were transformed into BL21(DE3)pLysS competent bacteria (Life Technologies) and grown in LB supplemented with 100 μg of ampicillin (Sigma-Aldrich) and 25 μg/mL chloramphenicol (Sigma-Aldrich). Transformants were induced with 0.4 mmol/L isopropyl-β-d-thiogalactopyranoside (Promega, Madison, Wis) for 12 hours at 37°C to induce recombinant knob protein. Recombinant knob protein was purified from cell lysates by using cOmplete His-Tag Purification Resin (Roche Life Sciences, Indianapolis, Ind) and passed through Detoxi-Gel Endotoxin Removing Gel (Thermo Fisher Scientific), according to the manufacturer's instructions. Residual endotoxin content was determined by using Limulus Amebocyte Lysate PYROGENT Plus (sensitivity = 0.06 EU/mL; Lonza, Walkersville, Md) and found to be less than detectable levels.

Conditions for qPCR

qPCR was performed with iTaq Universal SYBR Green Supermix 200 (Bio-Rad Laboratories). The reaction was carried out in an iQ 5 Multicolor Real-Time PCR Detection System (Bio-Rad Laboratories). The relative amount of target mRNA was calculated based on its threshold cycle, as suggested by the software (iQ 5 Optical System software), in comparison with the threshold cycle of the housekeeping gene β-actin. After denaturation, conditions for gene expression were as follows: 50 cycles of 30 seconds at 95°C followed by 30 seconds at 60°C each cycle for most genes. For murine *Tslp*, 60 cycles of amplification with 30 seconds at 95°C followed by 30 seconds at 54°C each cycle were performed; for murine *Il25*, 60 cycles with 30 seconds at 92°C followed by 30 seconds at 52°C each cycle were performed; and for murine *Il33*, 55 cycles with 30 seconds at 92°C followed by 30 seconds at 56°C each cycle were performed.

Immunohistology

For all immunohistologic analysis, tissue sections were subjected to antigen retrieval, followed by quenching of endogenous peroxidase activity before staining with specific antibodies. Sections were briefly counterstained (for 5 seconds) with hematoxylin. Appropriate VECTASTAIN ABC Kits using biotinylated secondary antibodies (Vector Laboratories, Burlingame, Calif) and the Peroxidase AEC (3-amino-9-ethylcarbazole) substrate kit (Vector Laboratories) were used for detection. Stained slides were examined with a Nikon Microphot EPI-FL microscope (Nikon, Tokyo, Japan), and images were captured with an Olympus DP71 camera (Olympus, Center Valley, Pa). For quantitation of eosinophils, MBP^+ cells in randomly selected nonoverlapping microscopic fields were counted at ×400 magnification (12 ± 2 fields per slide for OVA groups and 5 fields per slide for control groups). For quantitation of E-cadherin and occludin, positively stained areas in the epithelium of all similarly sized airways with total basement membrane length in the range of 550 to 650 μm were quantitated for each mouse.

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TABLE E1. List of primers

Gene	Forward primer	Reverse primer	Reference
Eotaxin-1 (mouse)	AGAGCTCCACAGCGCTTCT	GCAGGAAGTTGGGATGGAG	E1
Eotaxin-2 (mouse)	GCAGCATCTGTCCCAAGG	GCAGCTTGGGGTCAGTACA	E1
E-cadherin (human)	CCCAATAGATCTCCCTTCACAG	CCACCTCTAAGGCCATCTTTG	E2
Occludin (human)	TCAGGGAATATCCACCTATCACTTCAG	CATCAGCAGCAGCCATGTACTCTTCAC	E3
ZO-1 (human)	CGGTCCTCTGAGCCTGTAAG	GGATCTACATGCGACGACAA	E4
ICAM-1 (human)	CTGCAGACAGTGACCATC	GTCCAGTTTCCCGGACAA	E5
TSLP (mouse)	CGGATGGGGCTAACTTACA	TCCTCGATTTGCTCGAACTT	E6
IL-25 (mouse)	CAGCAAAGAGCAAGAACC	CCCTGTCCAACTCATAGC	E7
IL-33 (mouse)	CAATCAGGCGACGGTGTGGATGG	TCCGGAGGCGAGACGTCACC	E8
TARC (mouse)	ATGAGGTCACTTCAGATGCT	ATGTTTGTCTTTGGGGTCTG	E9
β-Actin (human)	AGGCACCAGGGCGTGAT	TCGTCCCAGTTGGTGACGAT	E10
β-Actin (mouse)	GGTCATCACTATTGGCAACG	ACGGATGTCAACGTCACACT	E11

ICAM-1, Intercellular adhesion molecule 1; *ZO-1*, zonula occludens 1.

TABLE E2. Effect of knob on cell viability

Treatment	Cell viability based on Trypan Blue exclusion (% mean ± SEM)
Untreated (culture medium alone)	98.08 ± 0.9
Knob (0.25 μg/mL)	94.38 ± 1.73