Supplementary Methods

Histopathologic Examination and Immunohistochemistry

Sections were stained with H&E and Alcian blue (pH 2.5) for histologic examination. For immunohistochemistry, deparaffinized sections were rehydrated and antigens were retrieved using Target Retrieval solution (DakoCytomation, Glostrup, Denmark) in a 2100 retriever (PickCell Laboratories, Amsterdam, The Netherlands).

For immunohistochemistry using anti-TFF2/SP, sections were blocked using blocking serum provided in the HistoMouse staining kit (Zymed, San Francisco, CA). Sections were incubated with murine monoclonal immunoglobulin (Ig) M anti-TFF2/SP (1:100; a gift from Sir Nicholas Wright, Cancer Research, London, England) overnight at 4°C. Indirect immunohistochemical detection was then performed through incubation with biotinylated secondary antibodies and alkaline phosphatase&ndash;conjugated streptavidin (Vectastain ABC Kit; Vector Laboratories, Burlingame, CA). Chromogen was developed with Vector Red (Vector Laboratories).

For immunohistochemistry using anti&ndash;H^+/K^+-adenosine triphosphatase, sections were blocked using blocking serum provided in the mouse on mouse (MOM) staining kit (Vector Laboratories). Sections were incubated with a primary murine monoclonal IgG anti&ndash;H^+/K^+- adenosine triphosphatase α-subunit (1:2000; a gift from Dr Adam Smolka, Medical University of South Carolina, Charleston, SC) overnight at 4°C. Indirect immunohistochemical detection was then performed through incubation with biotinylated secondary antibodies and alkaline phosphatase&ndash;conjugated streptavidin (Vectastain ABC Kit; Vector Laboratories). Chromogen was developed with Vector Red (Vector Laboratories).

For immunohistochemistry using anti-TFF3, anti&ndash;Pdx-1, anti&ndash;Cdx-2, and anti-Ki67, sections were blocked using normal serum provided in the Vectastain Kit (Vector Laboratories) and then incubated with the primary antibody overnight at 4°C. Immunostaining was performed with rabbit polyclonal anti-TFF3 (1:500; a gift from Dan Podolsky, Harvard Medical School, Boston, MA) or guinea pig anti&ndash;Pdx-1 (1:5000; a gift from Dr Christopher Wright, Vanderbilt University, Nashville, TN), mouse monoclonal anti&ndash;CDX-2 (1:100; BioGenex, San Ramon, CA), or rat monoclonal anti-Ki67 (1:200; TEC-3; DakoCytomation). For immunohistochemistry with detection with diaminobenzidine, the sections were incubated with biotinylated secondary antibody followed by horseradish peroxidase&ndash;conjugated streptavidin. Chromogen was developed with diaminobenzidine (Biogenex, San Ramon, CA).

For all immunostaining, the sections were counterstained with Mayer's hematoxylin and mounted. Sections were viewed and photographed on a Zeiss Axiophot bright-field microscope equipped with an Axiovision digital imaging system (Carl Zeiss, Thornwood, NJ).

Immunofluorescence

For immunofluorescence staining of β-catenin, deparaffinized sections were blocked with blocking serum provided in the MOM staining kit (Vector Laboratories) and incubated with murine monoclonal anti&ndash;β-catenin (1:500) overnight at 4°C, followed by incubation with Alexa488 goat anti-mouse IgG (Invitrogen, Carlsbad, CA). After washing with phosphate-buffered saline, sections were mounted using ProLong Gold Antifade Reagent with 4,6-diamino-2-phenylindole (Invitrogen) for nuclear counterstain and mounting medium.

For double staining with anti-TFF2/SP and anti-TFF3, deparaffinized sections were blocked with blocking serum provided in the MOM staining kit (Vector Laboratories) and incubated with anti-TFF2/SP (1:100) and anti-TFF3 (1:500) at the same time overnight at 4°C, followed by incubation with Cy3-labeled goat anti-mouse IgM (Jackson ImmunoResearch, West Grove, PA) and Alexa488 goat anti-rabbit IgG (Invitrogen). After washing with phosphate-buffered saline, sections were mounted as described previously with 4,6-diamino-2-phenylindole.

For double staining with anti-TFF2/SP and anti-MUC2, deparaffinized sections were blocked with blocking serum provided in the MOM staining kit (Vector Laboratories) and incubated with anti-TFF2/SP (1:100) and anti-MUC2 (1:500; Santa Cruz Biotechnology, Santa Cruz, CA) at the same time overnight at 4°C, followed by incubation with Cy3-labeled goat anti-mouse IgM (Jackson ImmunoResearch) and Alexa488 goat anti-rabbit IgG (Invitrogen). After washing with phosphate-buffered saline, sections were mounted as previously described.

All of the sections were viewed and photographed on a Zeiss Axiophot fluorescence microscope, and digital images were captured using a SPOT digital charge-coupled device camera (Carl Zeiss).

Cytokine Measurement

Sera collected from 10-month-old TGF-α and AR-null mice were analyzed for Th1 cytokines (interferon gamma, IL-2, and TNF-α) and Th2 cytokines (IL-4 and IL-5) using the Cytometric Bead Array assay (BD Biosciences, San Jose, CA) according to the manufacturer’s protocol. Briefly, sera were diluted 1:5 with assay diluent supplied by the manufacturer. Diluted sera were allowed to incubate with cytokine capture beads and PE detection reagent for 2 hours at room temperature. Cytokine-coated beads then were analyzed with a BD LSRII flow cytometer. Fluorescence data obtained from sera samples were compared with cytokine standards and analyzed with BD CBA software. The mean concentrations of cytokines then were compared between TGF-α and AR-null mice.

Quantitative <CASE>*Reverse-Transcriptase*</CASE> *Polymerase Chain Reaction*

To analyze cytokine gene expression in stomach, we performed reverse-transcribed real-time polymerase chain reaction. Stomachs from 10-month-old animals were homogenized using the pellet pestle (Kimble Chase, Vineland, NJ) and RNA was extracted using the RNeasy Mini Kit (Qiagen, Valencia, CA) according to the manufacturer’s protocol. Primers and probes for real-time quantitative polymerase chain reaction were purchased from Applied Biosystems (Foster City, CA) to measure Th1 or Th2 cytokine transcript levels based on GenBank sequences for murine glyceraldehyde-3-phosphate dehydrogenase, interferon gamma, TNF-α, and IL-2, IL-4, and IL-5. Probes were labeled at the 5&prime; end with 6-carboxyfluorescein and at the 3&prime; end with the nonfluorescent quencher Blackhole Quencher 1 (Operon Biotechnologies, Huntsville, AL). Reverse-transcribed real-time polymerase chain reaction was performed using Quantitect Probe RT-PCR kit (Qiagen) and a Smart Cycler II (Cepheid, Sunnyvale, CA) using 5 μL of extracted RNA. The parameters used were 1 cycle of 50°C for 30 minutes, 1 cycle of 95°C for 10 minutes, and 45 cycles of 95°C for 15 seconds and 60°C for 1 minute. Relative amounts of cytokine gene transcripts expressed were normalized to those of the glyceraldehyde-3-phosphate dehydrogenase housekeeping gene, and WT mice were used as baseline controls. Differences in RNA levels were computed using the ΔΔCt method, comparing with WT mice.

Statistical Analysis

The data were analyzed with the JMP software package (version 4.0; SAS Institute, Cary, NC). Cytokine levels were compared with analysis of variance followed by post hoc analysis of significant means by Dunnett's test. For all comparisons, *P* values less than .05 were considered statistically significant.