METHODS

Reagents

Recombinant human TNF, IL-1β, IL-4, IL-6, IL-10, IL-13, IL-17, IFN-γ, IFN-λ1, oncostatin M (OSM), and TGF-β were purchased from R&D systems (Minneapolis, Minn). Peptidoglycan from *Staphylococcus aureus*, polyinosine-polycytidylic acid (dsRNA), LPS from *Escherichia coli*, serotype 0111:B4, recombinant flagellin from *Salmonella typhimurium*, and IFN-β were purchased from InvivoGen (San Diego, Calif).

Cell culture

Human primary nasal epithelial cells (PNECs) were collected from the inferior turbinate or uncinate tissue by curettage with a Rhinoprobe (Arlington Scientific, Inc, Springville, Utah) under a Northwestern University Feinberg School of Medicine Institutional Review Board–approved human subject research protocol. PNECs were maintained in serum-free bronchial epithelial cell growth medium (BEGM; Cambrex, Walkersville, Md). PNECs were plated in 24-well culture plates coated with collagen (Vitrogen; Collagen Biomaterials, Palo Alto, Calif). Before stimulation, PNECs were cultured in BEGM without hydrocortisone for at least 36 hours. PNECs were stimulated with 10 μg/mL peptidoglycan, 25 μg/mL dsRNA, 1 μg/mL LPS, 10 ng/mL flagellin, 100 ng/mL TNF, 100 ng/mL IL-1β, 100 ng/mL IL-4, 100 ng/mL IL-6, 100 ng/mL IL-10, 100 ng/mL IL-13, 100 ng/mL IL-17, 1000 IU/mL IFN-β, 10 ng/mL IFN-γ, 100 ng/mL IFN-λ1, 100 ng/mL oncostatin M, or 100 ng/mL TGF-β for 6, 24, and 72 hours.

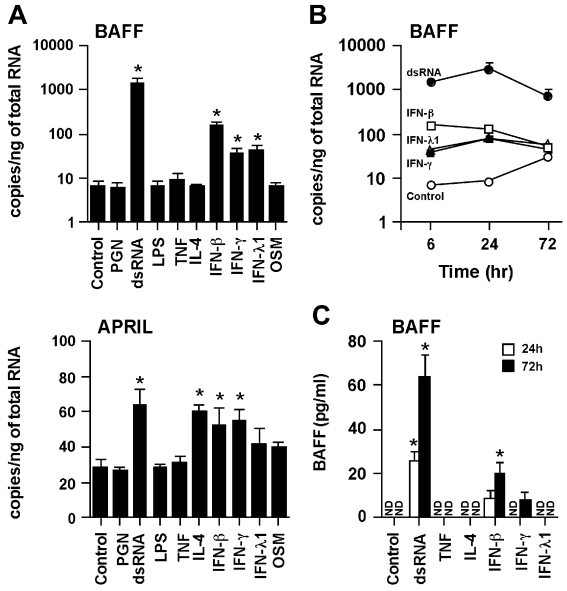

FIG E1. Expression of BAFF and APRIL in nasal epithelial cells. **A,** PNECs were incubated for 6 hours with 10 μg/mL peptidoglycan *(PGN)*, 25 μg/mL dsRNA, 1 μg/mL LPS, 100 ng/mL TNF, 100 ng/mL IL-4, 1000 U/mL IFN-β, 10 ng/mL IFN-γ, 100 ng/mL IFN-λ1, and 100 ng/mL oncostatin M *(OSM)* as indicated, and then mRNA was extracted and analyzed for BAFF and APRIL using real-time PCR. The mRNA expression levels were normalized to the median expression of the housekeeping gene, ACTB. **B,** PNECs were incubated with dsRNA, 1000 U/mL IFN-β, 10 ng/mL IFN-γ, 100 ng/mL IFN-λ1, or vehicle control (medium) for 6 to 72 hours, and then expression of mRNA for BAFF was analyzed by real-time PCR. **C,** Detection of BAFF protein by ELISA in the culture supernatant of PNECs stimulated with dsRNA and cytokines for 24 or 72 hours. Results shown are means ± SEMs of 4 to 7 independent experiments. *ND,* Not detectable. **P* < .05.