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Short Communication

# Interleukin-27 inhibits phagosomal acidification by blocking vacuolar ATPases

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ABSTRACT

Interleukin (IL)-27 is a unique cytokine that has a dual role in immune responses. It was originally described to promote Th1 differentiation but also suppresses inflammation by inhibiting these and other inflammatory T cell subsets. Inhibition of inflammatory activity in macrophages has also been reported. These reports have largely focused on cytokine profiles or signaling mechanisms. To date, there have been no reports of how IL-27 may directly influence cellular mechanisms that operate to control microbial growth. Formation of a phagolysosome that acquires antimicrobial properties is an essential step for destruction of pathogens or pathogen-derived materials that are internalized by macrophages. Here we report that IL-27 has a profound influence on this critical innate immunity pathway. Treatment of human macrophages with IL-27 interferes with the acidification of phagosomes by reducing protein levels of V-ATPase and impairs control of bacterial pathogens.



## 1. Introduction

Interleukin (IL)-27 is a member of the IL-6 sub-family of type I cytokines. IL-27 is produced by antigen presenting cells in response to a variety of activation stimuli, notably microbial-derived products [1,2]. IL-27 is known to promote $T_H1$ differentiation [3]. However, IL-27 inhibits differentiation of $T_H$-17 cells and IL-17 production [4]. Murine and human macrophage inflammatory signaling and cytokine production is diminished in response to IL-27 [4]. Mice deficient in the IL-27 receptor are unable to control inflammation during chronic infection [5,6]. Thus, while IL-27 has the ability to both enhance and suppress inflammation, the latter seems to dominate.

In the endosomal/lysosomal trafficking pathway, the phagosome fuses with endosomes that mature to lysosomes to mediate the clearance of microbial pathogens in macrophages [7]. IFN-γ promotes lysosomal fusion with endosomes and acidification [8]. Activated macrophages acidify phagosomes or endosomes by vacuolar $H^+$-ATPases (V-ATPase) [9]. These multisubunit protein complexes transport protons across membranes by hydrolyzing ATP to acidify endosomal compartments [10]. Here, we are showing for the first time that IL-27 decreases lysosomal acidification in human macrophages by influencing the expression of V-ATPase.

## 2. Materials and methods

### 2.1. Cell culture

Human buffy coats were purchased from the New York Blood Center (New York, NY). Eligible donors were 16 years of age or older, at least 110 lb, and in good physical health. The donor samples were anonymous and deidentifed. Monocytes were isolated by successive density gradient centrifugation as described previously [2]. Monocytes were differentiated to macrophages in DMEM supplemented with 2 mM glutamine, 25 mM HEPES, 20% FBS, and 10% human serum AB and incubated at 37 °C with 5% $CO_2$ for 7 days. The cells were washed with PBS and plated onto new culture dishes in DMEM supplemented with 1% human serum, 2 mM glutamine, and 25 mM HEPES.

### 2.2. Bacterial infection and gentamycin protection assay

*Pseudomonas aeruginosa* 1244 and *Staphylococcus aureus* RN6390 were kindly provided by Dr. Joseph Horzempa (West Liberty University) and Dr. Mark Hart (University of North Texas Health Science Center), respectively. The bacteria were grown overnight in Tryptic Soy broth at 37 °C and washed in PBS. The bacteria were adjusted to $1 \times 10^8$ CFU/ml using a spectrophotometer ($OD_{590}$ = 0.140: *P. aeruginosa*, $OD_{600}$ = 0.4: *S. aureus*). Macrophages were pretreated with IL-27 (30 ng/ml), bafilomycin (100 nM), or left untreated and then incubated with bacteria at an MOI of 10 for 4 h at 37 °C with 5% $CO_2$. At this time gentamycin (100 μg/ml) was included in the cultures. For the 24 h time point, gentamycin was maintained in the culture for an additional 20 h. At each

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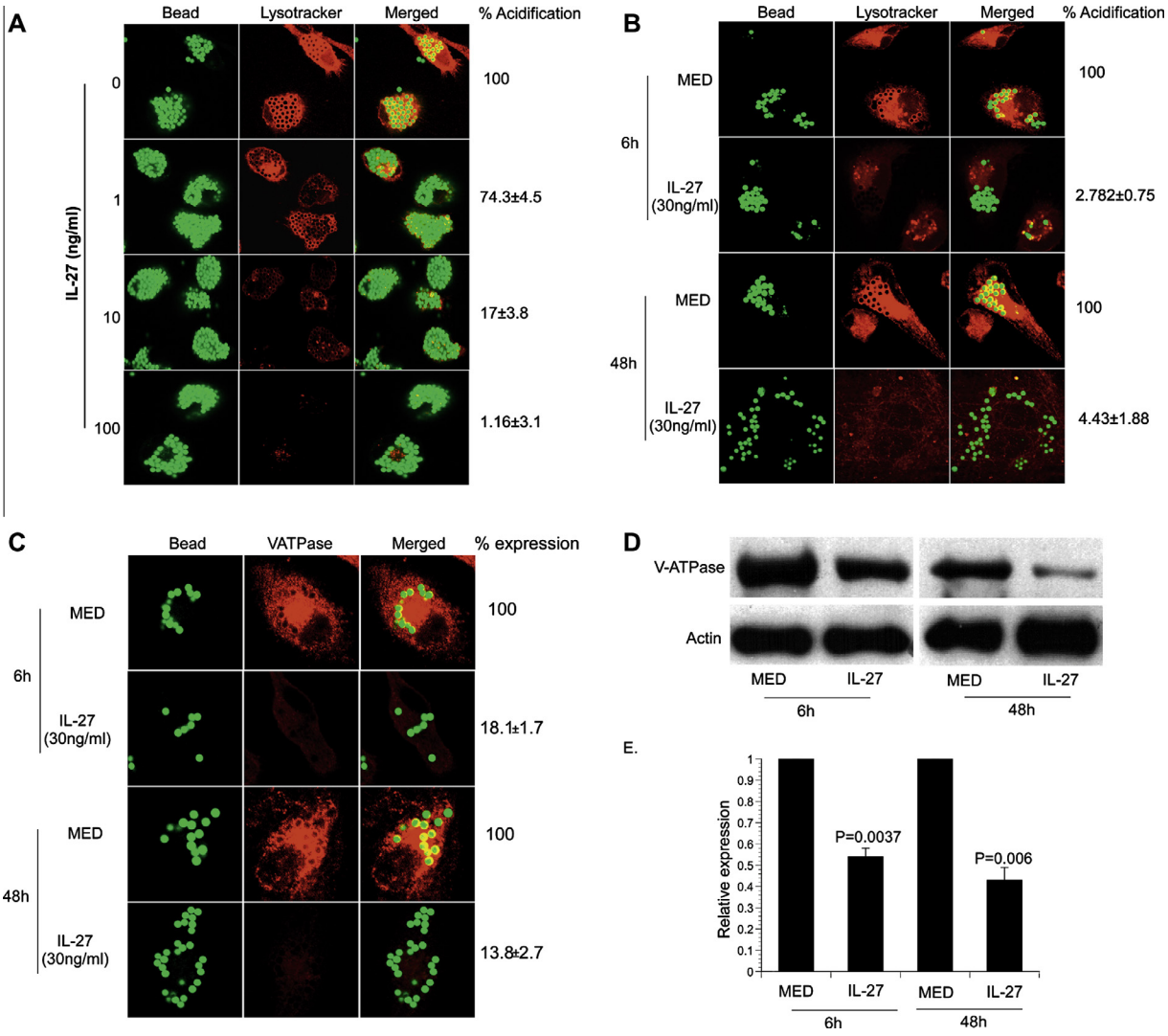


**Fig. 1.** IL-27 decreases the acidification of latex bead compartments by reducing expression of V-ATPase. Macrophages were either untreated (0 or MED) or treated with IL-27 as indicated. (A, B, and C) Cells were subjected to yellow-green fluorescent labeled latex beads for an additional 6 or 48 h as indicated. (A and B) Acidified lysosomes (red) were stained by Lysotracker (200 nM) following latex bead uptake. The images shown are from an individual experiment representative of three. The MFI obtained from untreated macrophages was set to 100%. All other conditions were expressed relative to this value. The values presented here are combined results from two (A) or three independent experiments (B). (C) Macrophages were stained with anti-V-ATPase H antibody as described. Labeled proteins were detected with anti-mouse IgG conjugated with Alexa-568 (red). Representative images from four experiments are shown. Percent expression of the protein was calculated as is described in Section 2. (D) Cell lysates were prepared at indicated times for immunoblot analysis. An image representative of three experiments is shown, and (E) The ratio of V-ATPase/actin band intensity was expressed relative to medium alone for three combined experiments. A Student's *t* test was used to compare ratios from the IL-27-treated group with the control (MED) at each time point in the 95% confidence interval.

end point, culture supernatants were removed and macrophages permeabilized with 1% saponin to release bacteria. Serial dilutions were plated on Tryptic Soy agar and incubated overnight at 37 °C.

### 2.3. Fluorescent bead uptake in macrophages

Human macrophages were cultivated in 24-well plates ($2 \times 10^5$/well). Yellow-green fluorescent- labeled 2 μm latex beads (~10 beads/macrophage; Sigma–Aldrich) were supplied to macrophages treated with or without IL-27 (eBioscience) or bafilomycin (Sigma). The bead conjugated fluorophore is stable at acidic pH.

### 2.4. Analysis of lysosomal acidification and V-ATPASE immunolabeling

At the indicated time point, macrophages were treated with Lysotracker DND-99 Red (Life Technologies) for 45 min and then fixed with PBS that contained 4% paraformaldehyde (PFA). The slides were examined using a Zeiss Meta 510 laser scanning confocal microscope. A total of ten fields containing 10–20 macrophages per field were examined in each experiment. The mean fluorescent intensity (MFI) for each macrophage was calculated using Image J software. For immunostaining, mouse anti-V1-ATPase H antibody (Santa Cruz Biotechnology) was visualized by anti-mouse-Alexafluor 588-conjugated secondary antibody. The percent V-ATPase expression was analyzed by MFI similar to as described above with approximately five macrophages per field. Protein lysates for immunoblots were prepared in parallel with imaging experiments by standard techniques. The same primary antibody was revealed on nitrocellulose by anti-mouse HRP.

### 2.5. Statistical analysis

A Student's *t* test was used to determine statistical significance in the 95% confidence interval ($P < 0.05$).