Human antimicrobial Th17 cells produce IL-26 in response to IL-1β

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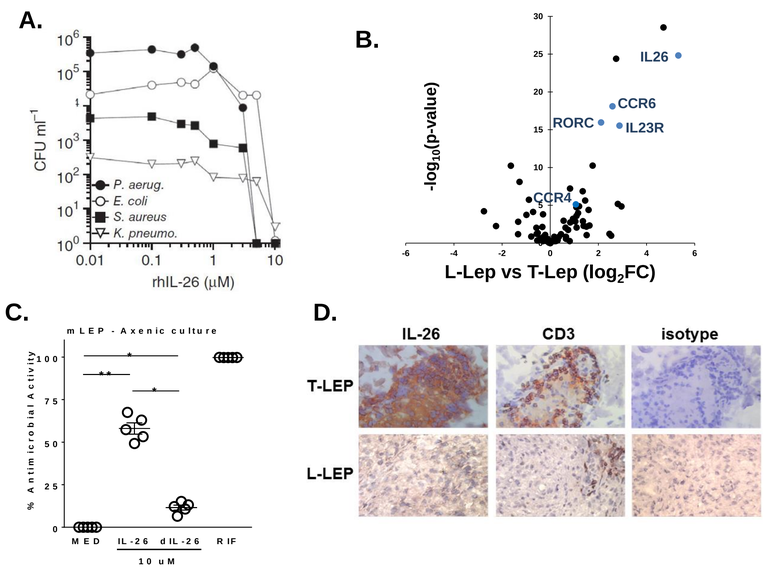
ABSTRACT

Th17 cells play a fundamental role in both immunity and autoimmunity at mucosal surfaces, including skin. Recent work has implicated the Th17 cytokine IL-26 as both directly antimicrobial to extracellular and intracellular bacteria, including *Mycobacterium leprae*, the causative agent of leprosy. IL-26 protein expression was greater in skin lesions from patients with the resistant vs. progressive form of the disease. To understand the mechanism for IL-26 induction, we examined the kinetics of IL-26 secretion by PBMCs exposed to *Mycobacterium leprae* sonicate, which revealed induction at 3 hours, reminiscent of an innate response. Pattern recognition receptor ligands induced IL-26 secretion from PBMC, which was mediated by IL-1β. Recombinant IL-1β alone was sufficient to stimulate IL-26 release from PBMC and memory CD4⁺ T cells in the absence of T cell receptor (TCR) activation. IL-1β stimulated IL-26 secretion more rapidly from memory CD4⁺ T cells as compared to TCR activation with crosslinking antibodies. Further investigation revealed that the majority of IL-1b induced IL-26 was form memory Th17 cells that expressed IL-1R1, and not from Th1 or Th2 cells. IL-1β stimulation did not lead to secretion of other Th17 cytokines, unlike TCR activation. RNA sequencing of IL-1β treated IL-1RI⁺ Th17 cells revealed enrichment for NF-κB regulated genes. Inhibition of NF-κB signaling with Bay 11-7082 abrogated IL-26 production in response to either stimulus. Finally, supernatants from IL-1β treated memory T cells showed antimicrobial activity against *E. coli* in an IL-26 dependent manner. Together, these results identify IL-1RI+ Th17 cells as an antimicrobial Th17 subpopulation with the ability to rapidly respond to IL-1β and induce IL-26 to kill extracellular bacteria.

BACKGROUND



A. Increasing amounts of IL-26 kills both gram positive and gram negative bacteria in axenic culture.[1]

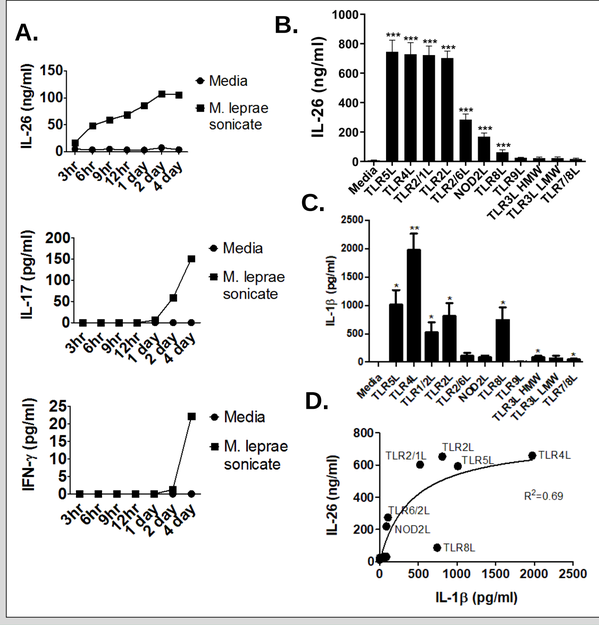
B. Sequencing of RNA extracted from either lepromatous leprosy (L-Lep) or tuberculoid leprosy (T-Lep) lesions shows differential expression of a variety of genes . Highlighted are several Th17 associated genes. Notably, IL-17A was not detected in the lesions, whereas IL-26 is highly differentially expressed.

C. IL-26 can kill *M. leprae* in axenic culture. Heat denaturation reduces the antimicrobial activity of IL-26. Rifampicin is shown as a positive control.[2]

D. The disease leprosy exists on a spectrum between two poles, self-limited tuberculoid leprosy and disseminated lepromatous leprosy. Immunohistochemical staining within lesions shows that IL-26 is abundant within tuberculoid leprosy lesions but not in lepromatous leprosy lesions.[2]

RESULTS

IL-26 is Secreted In Response to Innate Stimuli



A. PBMCs isolated from a patient with tuberculoid leprosy secrete IL-26 within hours, as compared to IL-17 and IFN-γ.

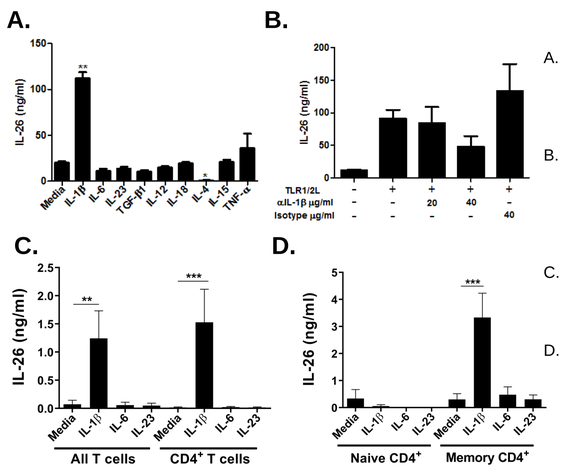
B. Incubating healthy donor PBMC with a variety of pattern recognition receptor (PRR) ligands leads to robust IL-26 secretion.

C. PRR ligands also induce IL-1β secretion from PBMCs.

D. Il-26 and IL-1β secretion are correlated after PRR ligand stimulation

RESULTS

IL-1β induces IL-26 secretion from PBMC and T cells



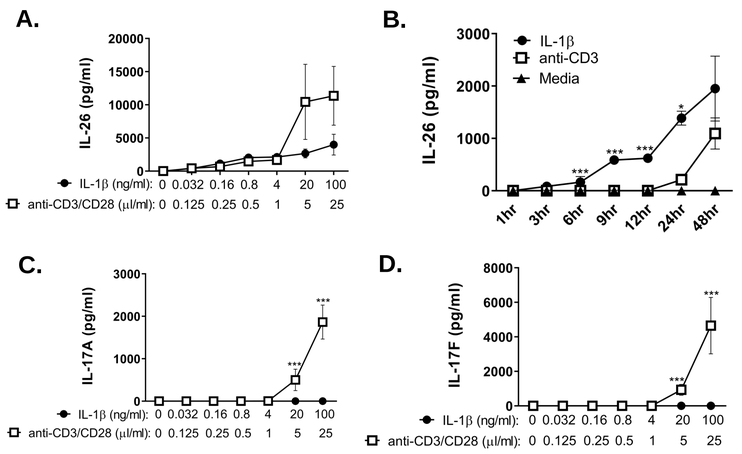
A. In PBMCs cultured for 48 hours with 20 ng/ml of various cytokines, only IL-1β induced IL-26 secretion.

B. Adding increasing amounts of α-IL-1β to PBMCs stimulated with TLR1/2L shows a trend to decreased detectable IL-26 by ELISA

C. IL-1β induces IL-26 secretion from CD3⁺ T cells and enriched CD4⁺ T cells.

D. Within the CD4⁺ T cells, IL-1β induces IL-26 secretion from the CD45RA⁻ memory cells

IL-26 secretion is faster with IL-1β vs TCR activation

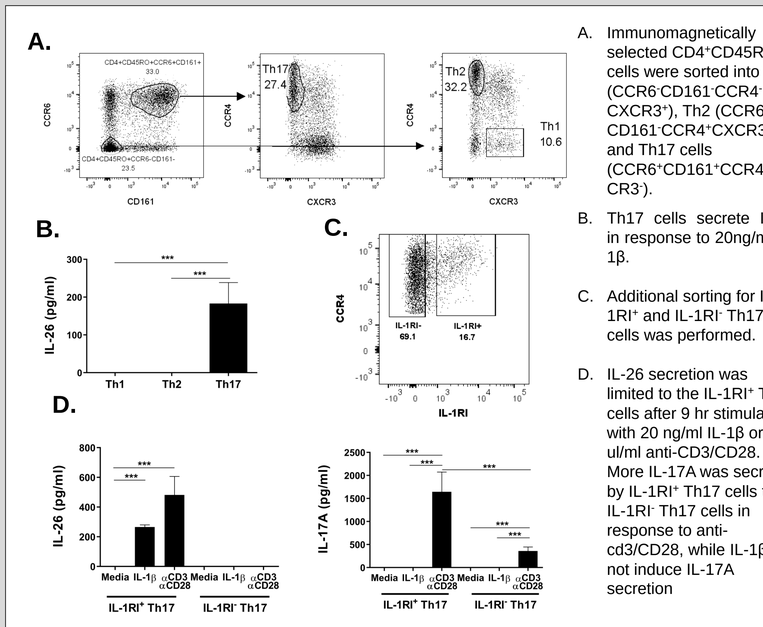


A. Dose titration of IL-1β and anti-CD3/CD28 solution

B. IL-1β induces secretion of IL-26 more rapidly than anti-CD3/CD28 antibodies.

C. IL-1β does not stimulate IL-17A (C), IL-17F (D), or IL-22 (E) secretion, unlike anti-CD3/CD28 stimulation.

IL-1RI⁺ Th17 cells secrete IL-26 in response to IL-1β



A. Immunomagnetically selected CD4⁺CD45RO⁺ cells were sorted into Th1 (CCR6⁻CD161⁻CCR4⁻CXCR3⁺), Th2 (CCR6⁻CD161⁻CCR4⁺CXCR3⁻), and Th17 cells (CCR6⁺CD161⁺CCR4⁺CXCR3⁻).

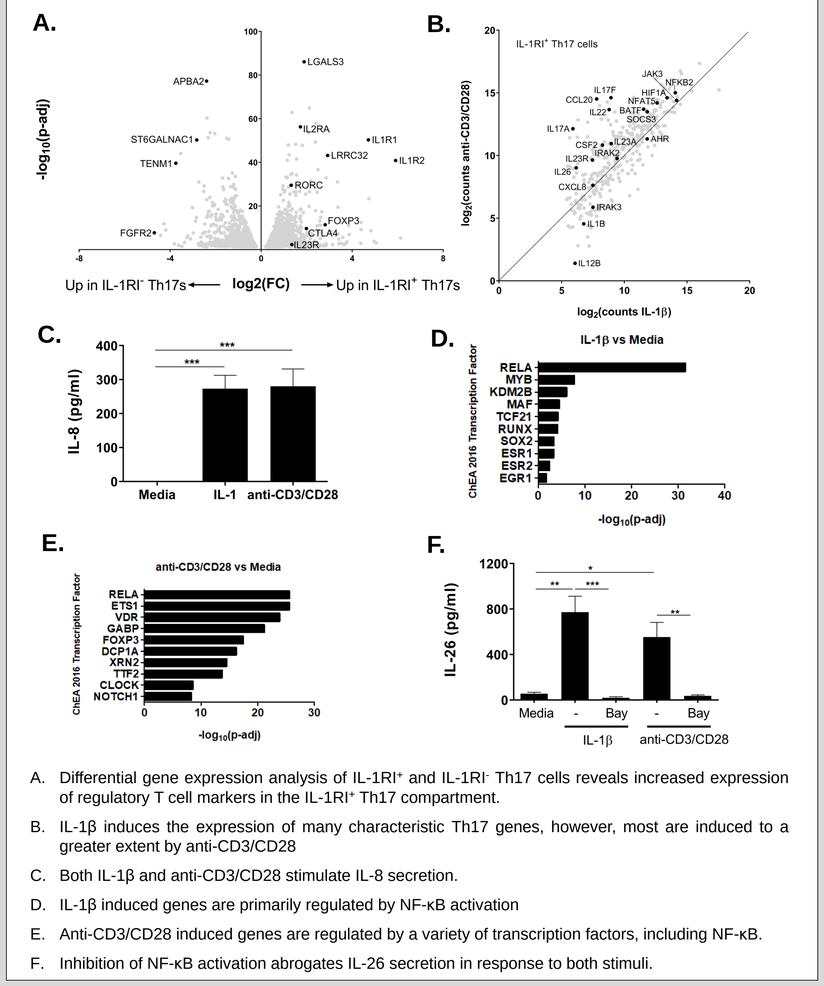
B. Th17 cells secrete IL-26 in response to 20ng/ml IL-1β.

C. Additional sorting for IL-1RI⁺ and IL-1RI⁻ Th17 cells was performed.

D. IL-26 secretion was limited to the IL-1RI⁺ Th17 cells after 9 hr stimulation with 20 ng/ml IL-1β or 5 ul/ml anti-CD3/CD28. More IL-17A was secreted by IL-1RI⁺ Th17 cells than IL-1RI⁻ Th17 cells in response to anti-cd3/CD28, while IL-1β did not induce IL-17A secretion

RESULTS

IL-1β induces NF-κB program in IL-1RI⁺ Th17 cells



A. Differential gene expression analysis of IL-1RI⁺ and IL-1RI⁻ Th17 cells reveals increased expression of regulatory T cell markers in the IL-1RI⁺ Th17 compartment.

B. IL-1β induces the expression of many characteristic Th17 genes, however, most are induced to a greater extent by anti-CD3/CD28

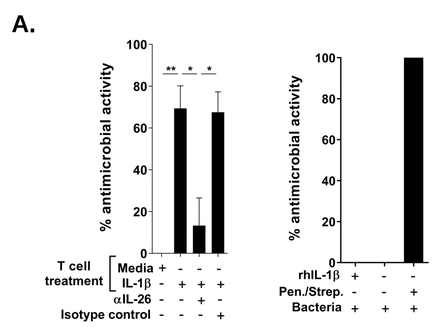
C. Both IL-1β and anti-CD3/CD28 stimulate IL-8 secretion.

D. IL-1β induced genes are primarily regulated by NF-κB activation

E. Anti-CD3/CD28 induced genes are regulated by a variety of transcription factors, including NF-κB.

F. Inhibition of NF-κB activation abrogates IL-26 secretion in response to both stimuli.

IL-1β treated T cell supernatants kill *E. coli*



A. Supernatants from memory CD4⁺ T cells treated with IL-1β exhibit IL-26 dependent antimicrobial activity against *E. coli*.

CONCLUSIONS & FUTURE DIRECTIONS

Conclusions:

- IL-26 is secreted in response to a variety of pattern recognition receptor ligands, particularly those that stimulate IL-1β secretion.
- IL-1β stimulates secretion of IL-26 from PBMCs, memory CD4⁺ T cells, and IL-1RI⁺ Th17 cells in the absence of TCR activation.
- Stimulation with IL-1β leads to faster IL-26 secretion than anti-CD3/CD28, but does not induce secretion of IL-17A, IL-17F, or IL-22.
- IL-1RI⁺ Th17 cells express more Treg markers, and IL-1β induces their expression of typical Th17 genes, but to a lesser extent than TCR activation.
- IL-26 secretion depends on NF-κB activation.
- This alternative pathway for Il-26 secretion via IL-1β leads to functional bacterial killing.

- **We plan to:**
- Investigate the kinetics of NF-κB activation with IL-1β or anti-CD3/CD28 activation to provide further mechanistic evidence for rapid IL-26 secretion.
- Determine whether IL-1RI⁺ Th17 cells are differentially present within tuberculoid and lepromatous leprosy lesions via immunohistochemistry.

References:

1. Meller S *et al.* (2015) Th17 cells promote microbial killing and innate immune sensing of DNA via interleukin 26. *Nat Immunol.* 16(9):970-9.
2. Dang AT *et al.* (2017) Th17 cell killing of intracellular pathogens mediated by IL-26. *In preparation.*