

and L33 have been placed in density. The A, P, and E sites were built into unambiguous density and found to be consistent with other bacterial structures. Notably, 1 EM density map contains an uncharged t-RNA molecule in the E site. The sites identified for current antibiotics are also well defined and interpretable. This 70S structural platform is suitable for structural analysis of antibiotic binding sites, especially for those antibiotics directed specifically against the enterococcal ribosome.

**Conclusion.** For the first time, the structure of the ribosome from the important human pathogen *Enterococcus faecalis* has been determined. The maps were obtained at high resolution and found to be suitable for antibiotic design. It is anticipated that the continued determination of the structures of ribosomes from pathogens will aid in the discovery of new treatments for infectious diseases.

**Disclosures.** All authors: No reported disclosures.

### 165. *Mycobacterium tuberculosis* Produces Molecules That Trigger Nociceptive Neurons to Activate Cough

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**Background.** A hallmark symptom of active pulmonary tuberculosis vital for disease transmission is cough. The current paradigm for tuberculosis-related cough is that it results from airway damage or irritation. However, there is limited experimental data to support this theory, and whether *Mycobacterium tuberculosis* (Mtb) induces cough to facilitate its own transmission has not been explored. The cough reflex is a complex and coordinated event involving both the nervous and musculoskeletal systems initiated by particulate or chemical molecules activating nociceptive neurons, which sense pain or irritation. This activation induces a signaling cascade ultimately resulting in a cough. Respiratory nociceptive neurons innervate the airway of humans and most mammals, and thus are poised to respond to noxious molecules to help protect the lung from damage. Because Mtb is a lung pathogen, cough is a primary mechanism of Mtb transmission, and respiratory nociceptive neurons activate cough, we hypothesized that Mtb produces molecules that stimulate cough, thereby facilitating its spread from infected to uninfected individuals.

**Methods.** We used an *in vitro* neuronal activation bioassay to fractionate, identify, and characterize Mtb cough-inducing molecules. We also measured cough *in vivo* in response to pure Mtb-derived cough molecules and during Mtb infection using a guinea pig model.

**Results.** We found that an acellular organic extract of Mtb triggers and activates nociceptive neurons *in vitro* with a neuronal response that is as robust as the response to capsaicin, an established nociceptive and cough-inducing molecule. Using analytical chemistry and our neuronal bioassay, we then isolated 2 molecules produced by Mtb that activate nociceptive neurons. Both the organic Mtb extract and purified molecules alone were sufficient to induce cough in a conscious guinea pig cough model. Finally guinea pigs infected with wild-type Mtb cough much more frequently than guinea pigs infected with Mtb strains unable to produce nociceptive molecules.

**Conclusion.** We conclude that Mtb produces molecules that activate nociceptive neurons and induce cough. These findings have significant implications for our understanding of Mtb transmission.

**Disclosures.** All authors: No reported disclosures.

### 166. TGF- $\beta$ Restricts T-cell IFN $\gamma$ Production in Pulmonary Tuberculous Granulomas

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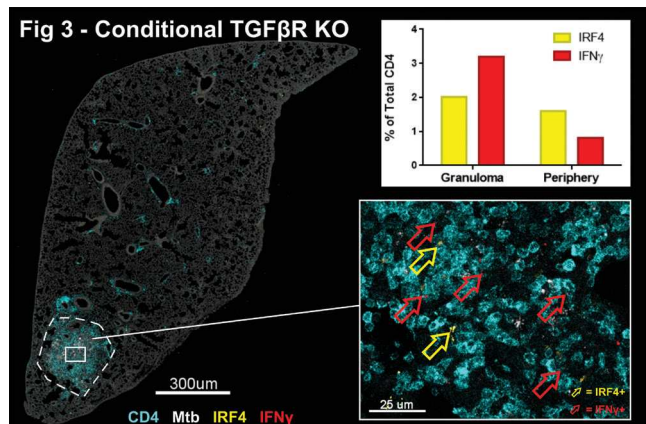
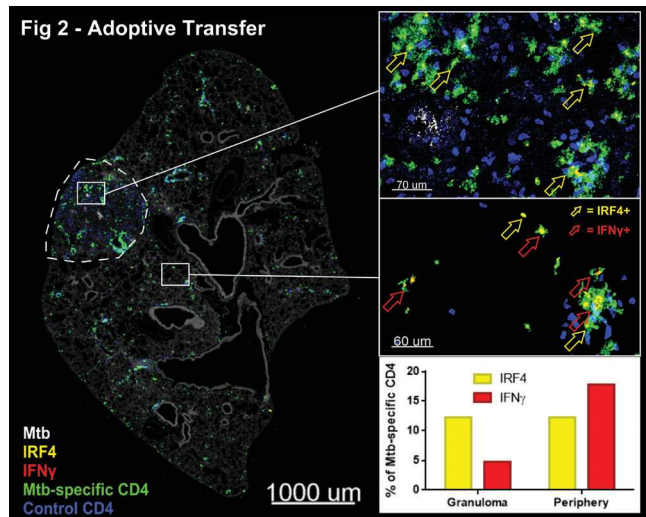
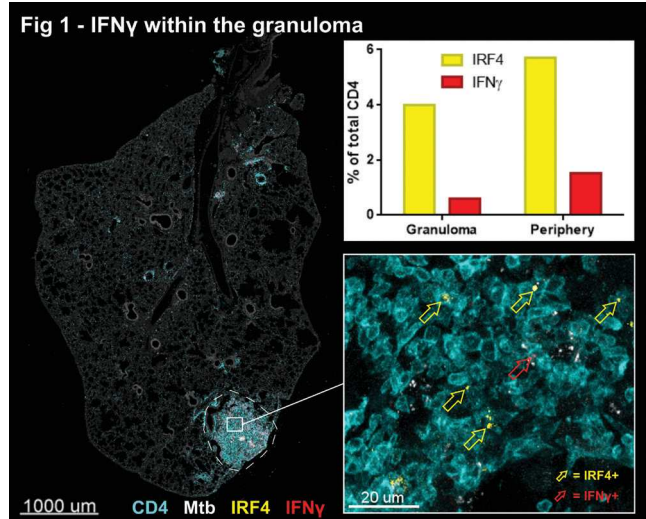
**Background.** IFN $\gamma$  production by CD4 T cells has been thought to be critical for immunity against *Mycobacterium tuberculosis* (Mtb); however, recent studies show that IFN $\gamma$ -producing CD4 T cells are more effective at preventing dissemination than controlling Mtb in the lung. Because optimal control of Mtb infection requires direct interactions between CD4 T cells and Mtb-infected cells presenting cognate antigen on MHCII, we sought to determine the location of CD4 T-cell antigen recognition and IFN $\gamma$  production in the Mtb-infected lung.

**Methods.** We infected mice with an ultra-low dose (ULD) of Mtb (1–3 CFU), a model developed in our laboratory which results in well-circumscribed granulomas that recapitulate many features of human Mtb granulomas. Using immunohistochemistry and quantitative imaging, we examined their lungs 35 days later for phenotypic and spatial analysis of T-cell receptor (TCR) signaling (using IRF4) and IFN $\gamma$  production. We tested the antigen specificity of these responses with an adoptive transfer of both Mtb-specific and OVA-specific control CD4 T cells into ULD Mtb-infected mice. To assess the role of TGF $\beta$  signaling on T-cell localization and function, we performed the same analysis in mice lacking the TGF $\beta$  receptor (TGF $\beta$ R) on T cells.

**Results.** Within Mtb-infected lungs, many T cells localize near Mtb cells and undergo TCR signaling. Despite this, we found very few cells producing IFN $\gamma$  within the granuloma (Figure 1). In our adoptive transfer experiment, both cell types

infiltrated the granuloma. The Mtb-specific, but not OVA-specific, T cells had active TCR engagement though only a small fraction of these cells produced IFN $\gamma$ , and this IFN $\gamma$  was diminished near Mtb (Figure 2). Conversely, in the TGF $\beta$ R conditional knockout, we found increased IFN $\gamma$  production that was highest within the granuloma (Figure 3).

**Conclusion.** Despite ongoing TCR stimulation in T cells, IFN $\gamma$  production is restricted in areas where cognate interactions are most likely to occur. TGF $\beta$  plays a critical role in mediating this effect, as T cells lacking the receptor can produce more IFN $\gamma$  near infected cells. These findings help explain why IFN $\gamma$ -producing T cells have limited capacity to control pulmonary Mtb infection and could guide new strategies for vaccine and immunotherapeutic development.



**Disclosures.** All authors: No reported disclosures.