Development of an assay to quantify mycobacterial lipid antigen-specific T cell receptors in human tissues and blood
Burden and diagnosis of mycobacterial diseases

Estimated TB incidence rates, 2019

T cell receptors repertoires are shaped by the history of an individual’s antigen exposure

Adapted from Figure 4-10 Immunobiology, 7ed (Garland Science 2008)
Adapted from Emerson et al. Nat. gen. 2017

**TCR-based diagnostics**

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**Coronavirus (COVID-19) Update: FDA Authorizes Adaptive Biotechnologies T-Detect COVID Test**

For Immediate Release: March 05, 2021

Today, the U.S. Food and Drug Administration issued an emergency use authorization (EUA) for the T-Detect COVID Test developed by Adaptive Biotechnologies. The T-Detect COVID Test is a next generation sequencing based (NGS) test to aid in identifying individuals with an adaptive T cell immune response to SARS-CoV-2, indicating recent or prior infection with SARS-CoV-2.
T cells can recognize lipid antigen bound to CD1, which is highly conserved among humans.

There are lipid-specific T cells with partially invariant TCRs shared among most humans.

DeWitt et al. *imm.* 2018
Germline-encoded mycolyl lipid-reactive (GEM)-T cells have a partially invariant TCRα

TRAV1-2 TRAJ9 Constant chain

Van Rhijn et al. *Nature Immunology*. 2013
Design a PCR assay to quantify GEM - TCR
Assay primer design and validation on plasmid DNA

TRAV1-2  TRAJ9  Constant chain

GEM-TCRα fwd  TCRα Control fwd
GEM-TCRα rev  TCRα Control rev

GEM-TCRα plasmid DNA
Quantification of GEM - TCR from T cells

GEM-TCRα expression normalized to total TCRα
Testing in clinical cohorts

GEM-T cells recognize glucose monomycolate, which is produced during infection by M.Tb and M.Leprae
Leprosy Cohort: Individuals in Nepal recruited based on clinical presentation

1. Extract RNA
2. Pathology to determine if tuberculoid vs lepromatous vs not leprosy
TB cohort: Individuals in S. Africa tested for 

*M. Tb* exposure or with active TB disease

Content source: [Centers for Disease Control and Prevention, Division of Tuberculosis Elimination](https://www.cdc.gov)
Summary

- Designed a simple PCR assay to quantify expression of the GEMTCRα
- Validated assay on plasmid DNA and GEM-TCR expressing T cell line
- Tested assay on two different cohorts
  - GEM-TCRα expression is able to be detected in almost all samples
  - T cell abundance is increased in dermal biopsies from individuals with leprosy compared to controls suggesting trafficking T cells to the sites of disease
  - Proportions of GEM-TCRα is unchanged between individuals with leprosy or TB compared to controls
Discussion and Future Work

- Glucose monomycolate is also present in nontuberculous mycobacteria and other related bacteria, so not entirely specific
  - May need to focus on lipids with more restricted expression such as sulfoglycolipids, which is only expressed by virulent mycobacteria
- Alternatively, may need a multiplex assay for better specificity, combining measurement of several mycobacterial lipid-specific TCRs
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