How does one get TB???

Transmission and Pathogenesis: Factors
- Source/environment
- Organism
- Host

Topics
- Transmission
- Infection: Sensitization and Containment
- “Latency:” The opportunity
- Reactivation: “Adult” TB disease
Transmission/Infection: Source

- Respiratory route – aerosol (most common)
  - Usually unsuspected, “infectious” TB
- Ingestion
  - Unpasteurized milk
  - Uncooked meat
**TB pathogenesis**

- TB virulence mechanisms
  - Entry to macrophage
  - Phagosomal failure to acidify
  - No phago-lysosomal fusion
  - Avoidance of apoptosis
  - Intracellular replication
  - Immune subversion: TNF-α, IFN-γ

- Host response
  - Phagocytosis
  - NO, ROS, Fe deprivation
  - Cytokines
  - Apoptosis
  - T cells: CMI & granuloma
  - Time Course: 8-10 weeks

**Initial Steps**

- APCs present antigens to T-cells complexed in the groove of MHC II (HLA DR, DP, DQ)
- Most DTH responses are elicited through TCR-β
  - Activation requires additional signaling via CD4/CD8 molecules and co-stimulation - not physically associated with TCR
  - For memory or effector T-cell activation, expression of inducible co-stimulator (ICOS)-L on APC that interacts with ICOS expressed on T cells is required
Cytokines and TB

- Family of secreted proteins that bind immune cells through specific signaling receptors
- IFNγ – key ingredient
  - High levels found at rxn sites
  - Direct injection induces inflammatory rxn
- Also: IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, G-CSF, GM-CSF, PGE2, MCP-1, TGF-β, IFN-β identified in lesions
- TNF-α
  - Injection also induces inflammation

TH1 versus TH2
Mutually Exclusive?

- TH1
  - IFN-γ: macrophage activation (DTH)
  - IL2, TNF-α
- TH2
  - IL4, 5, 6: B cell activation (humoral immunity, tolerance)
- Induction depends on DC-generated cytokines:
  - IL12 → TH1
  - IL10 → TH2

IFN-γ

- Produced by NK and TH1 cells
- Major activator of macrophage-monocyte lineage cells
  - Augments phagocytosis, respiratory burst
  - Upregulates MHC
- Upregulates T-cell activation markers (CD69, CD71), IL2Ra (CD25), HLA-DR
  - Promotes TH1 differentiation
- Suppresses TH2 differentiation
  - Downregulates TH2 cytokines – IL4, 5

TNF-α

- Produced by macrophages, TH1 (other cells)
- Induces macrophage, endothelial cell chemokines
- Up-regulates vascular endothelial cell adhesion molecules
- Induces inflammatory cytokines (IL1)
Interleukin 12

- Produced by APC
- Augments NK/T cell IFN-γ production
- Promotes TH1 cell-differentiation
- Enhances NK and CD8+ T cell cytolytic function

Primary TB in a Child

Containment
“Latency” of M. tuberculosis

- Environment of granuloma favors altered metabolism:
  - Low pO2
  - Reduced CHO
  - High Fat
- Replication time >>> 20hr.
- Loss of acid fast staining properties
- Mechanism(s) unknown
  - genetic switch?

Mantoux Test

The Standard Tuberculin Skin Test

0.1 mL 5 TU PPD

Type IV Hypersensitivity

- antigens
- T cell
- cellular infiltrate
- swelling

Cytokine secretion: INF-α, INF-γ, TNF-α, IFN-γ, etc.

Neutrophil, monocyte, eosinophil, macrophage

Recruitment, proliferation

Fig. 3. Frequency distribution of reactions to 5 TU PPD-5 among white male Indo. Navy recruits 17 to 21 yr. of age who gave no history of household contact with tuberculous. Adapted from 1951 to 1950. Patient P. Thomas J. A method for estimating the prevalence of tuberculous infection. Am. J. Epidemiol. 1970; 161: 301-322.
Tuberculosis Cases by Skin Test Results
Massachusetts, 2006

- **Not Done**: 13 (5%)
- **Not Significant**: 27 (10%)
- **Unknown**: 6 (2%)
- **Anergic**: 3 (1%)
- **Significant**: 210 (82%)

MDPH Bureau of Communicable Disease Control, ISIS
(n = 259)

Tuberculin Skin Test in HIV

- Insensitive in low-prevalence populations
- Reactivity varies with level of immunosuppression
  - In early HIV, reactivity is maintained
  - Smaller or no reaction in advanced HIV
- Cut point is reduced
- Positive reaction (≥ 5mm; U.S. standard) should raise suspicion for TB infection
- Anergy testing generally is unreliable

**Tuberculin Skin Test Responses**
**by CD4 Lymphocyte Count**


**“Boosting”**

- **Principle: Loss of Immune Memory**
  - Number of sensitized T-lymphocytes declines over time following initial sensitization
  - A TST administered will:
    1. Induce a reduced inflammatory response at site
    - This may be interpreted as a Negative TST (a false negative) and
  2. Recall prior sensitization – expanding clone of sensitized T-cells within 3-7d
  - A subsequent TST will reflect prior sensitization
  - Prior sensitization: Positive TST
- **Solution: 2-step testing** in selected persons
DTH: Laboratory Correlates

- Lymphocyte proliferation (blast transformation)
  - H3-thymidine uptake on stimulation with antigen, mitogen
- Mixed lymphocyte reaction (MLR)
  - Thymidine incorporation T-cell reaction to cell surface antigens
- Cytokine generation
  - QuantiFERONs
  - T Spot TB

Interferon-gamma Release Assays (IGRA)

- *in vitro* assays for Cell-Mediated Immunity to M. tuberculosis antigens
  - Utilize whole blood
  - Measure release of IFN-γ by circulating "effector" T-lymphocytes following stimulation with PPD, other antigens
- QuantiFERON-TB (PPD): approved by FDA 2001 as “... an aid to the diagnosis of TB infection.”
- QFT-Gold (specific antigens): FDA approved 2005
- QFT-Gold in-Tube: approved 2007
- T Spot-TB: approved 2008

IGRA Principles

Antigen recognition and secretion of IFNγ

QuantiFERON-TB GOLD IT Method

Stage 1 Whole Blood Culture

- Repurpse whole blood
- ESAT-6, CFP-10, TB7.7
- Mitogen
- Culture overnight at 37°C
- TB infected individuals respond by secreting IFN-γ

Stage 2 IFN-γ ELISA

- Harvest Plasma from above settled cells and incubate 120 min in ‘Sandwich’ ELISA
- Wash, add Substrate, incubate 30 min then stop reaction
- Measure OD and determine IFN-γ levels

*Shaken – or Stirred?*
### Species specificity of ESAT-6 & CFP-10

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<tr>
<th>Species specificity</th>
<th>ESAT</th>
<th>CFP</th>
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<td>Mycobacterium xenopi</td>
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### QFT-G in-Tube Sensitivity for LTBI?

- Tested by TST/QFT-G (n=400)
- QFT-positive (n=8-10)
- TST-positive (n=40)

What about Boosting? ???

### The Answer?

- **TBESC Study 19**
  - Study of QFT-G-IT in household contacts
  - To follow QFT-neg contacts for 1 yr
  - To assess acceptance of QFT result by contacts, re. perception of need for treatment
  - 2 national sites
  - SF, Chicago
- **Diel, et al.**
  - 601 household contacts x 103 wk
  - 243 (40%) TST +, 66 (11%) QFT-pos
  - 6 developed TB; all were QFT-pos, declined tx
  - **BUT:**
    - no indeterminates (in 292 tested w mitogen)
    - Small numbers

### Preventing TB: Treatment of Latent TB Infection

- Significant TST or IGRA test
- Active disease excluded
  - History, examination, CXR
- Consider coincident co-morbidities/medications
  - As affecting risks/benefits of treatment
- **Treatment**
  - Isoniazid (INH) 300mg/d x 9 mos (standard)
  - Rifampin 600mg/d x 4 mos
- Reduces risk of disease by >90%
Treatment of Latent TB Infection:  
*Research Horizon*

- New drug regimens  
  - *e.g.* TBTC Study 26 (9m INH vs 3m INH/RPT 1x/wk)
- New drug development  
  - Fluoroquinolones  
  - Diarylquinolines  
  - Others …
- Vaccine development: *Modulating the Host Response*  
  - To prevent infection  
  - To prevent disease in infected persons  
  - To use as adjuvant treatment in persons with disease

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**Accelerating the Decline:**  
*The Opportunity*

- 224 Active TB cases
- 300,000 to 600,000 persons have TB infection

Most TB in Massachusetts  
Results from reactivation of TB infection (Genotyping: CDC)

Massachusetts, 2010

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**Reactivation**

- Latency and active Tuberculosis  
  - A continuum?  
    - Immune Surveillance *versus* the mycobacteria  
    - Evidence for ongoing metabolism by organisms  
- Reactivation $\rightarrow$ Loss of containment  
  - Risk increases with age  
  - Usually involves lungs as primary site of disease  
  - Poorly understood factors  
    - HIV infection  
    - Steroids  
    - TNF inhibitors  
    - Role of IFN-$\gamma$ and other cytokines?
TBTC Study 26

- CDC-sponsored study of TLTBI in high-risk persons
  - Close contacts, recent converters, HIV, >2cm nodule
- 9 mos INH (270 doses; self-administered) vs 3 mos INH + Rifapentine once a week (12 doses; by DOT)
  - Safety and efficacy
- At 23 TBTC sites, US, Canada, and overseas
  - 8,000 subjects enrolled; 2 year follow-up
- Non-inferiority (re-)design

Can we extrapolate findings to other groups?