Mycobacterium avium paratuberculosis in Crohn’s: a study using tissue PCR, in situ PCR and interferon-γ responses

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Background

• *M. avium paratuberculosis* (MAP): emerging pathogen, cause of IBD in cattle and other animals
• Histopathological similarities between certain types of paratuberculosis (sheep paucibacillary) and Crohn’s disease
• Issues in proving link between MAP and Crohn’s:
  – Technical: accurate diagnostic tests
  – Conceptual: study design
    • Case definition/classification of Crohn’s disease
    • Appropriate controls
Hypothesis

- Humans are exposed to MAP
- A subset of exposure leads to infection
- A subset of infection presents as IBD
  - Either Crohn’s disease or Crohn’s-like disease
  - Conversely, all Crohn’s not necessarily due to MAP
Objectives

• Detect MAP DNA in tissue samples of excisional biopsies from Crohn’s and controls:
  – tissue PCR
  – *in situ* PCR

• Determine prevalence of immunologic reactivity to MAP in Crohn’s and controls:
  – cell-mediated immune response (IFN-γ assay) to Mycobacterial antigens (MAP and others)
Techniques used: MAP DNA

- **Tissue PCR (IS900 in extracted DNA):**
  - Paucibacillary paratuberculosis samples from sheep amplify for MAP and not TB
    - Sensitive: 12/12, acid-fast negative samples (<10,000 bact/mL)
    - Specific: IS900 does not amplify in TB samples
- **in situ PCR (IS900 on pathology slide):**
  - Sheep samples and BCG infected mouse tissue
    - Sensitivity: improved detection versus *in situ* hybridization
    - Specificity: again – MAP amplifies from paratuberculosis only
Techniques used: immune studies

• IFN-γ assay:
  – PBMCs from study subjects stimulated in vitro:
    • PHA (positive control)
    • MAP tuberculin (paratuberculin)
    • Other Mycobacterial antigens
  – IFN-γ expression measured by Q-RT-PCR
Tissue PCR: First 14 samples

IS900 for M. paratuberculosis (1/14)
In situ PCR: Sheep paratuberculosis
IFN-γ assay: First 60 samples
Provisional interpretation

- PCR: too early to judge
- In situ PCR: Promising modality, utility remains to be seen (stand-alone?, confirmatory?)
- Mycobacterial immunity: Exposure to *Mycobacterium avium* complex clearly common
- Cases vs. controls: Assays blinded to subject classification, do not know if any trends exist
Upcoming directions

• PCR: to complete ~100 by July 2005
• In situ PCR: to complete by summer 2005
• Mycobacterial immunity: Already studied ~60, to complete by May 2005
• Unblinding and analysis: summer 2005
• Blood stored on subjects for humoral studies and genetic analysis in the future