Target discovery for immunodiagnosis of invasive aspergillosis

Tom Kozel
University of Nevada School of Medicine
Antigens as targets for immunodiagnosis

■ Principle
  ▪ Microbial antigens are shed in vivo
  ▪ Antigens can be detected by immunoassay

■ Advantages of antigen detection diagnostics
  ▪ Readily adapted to point-of-care format
  ▪ If antigen is shed, may detect infection at peripheral sites
  ▪ Proven technology; simple; inexpensive
  ▪ Minimal sample preparation

■ Examples
  – Streptococcus pyogenes
  – E. coli 0157
  – Rotavirus
  – C. difficile
  – RSV
  – H. pylori
  – Encapsulated bacteria
  – C. neoformans
Formats for antigen immunoassay

- Latex agglutination
- Antigen capture (sandwich) ELISA
- Lateral flow immunochromatographic assay
- Microbead-based immunoassay
- Microarray-based immunoassay
Targeting **proteins** for immunodiagnosis

- Many/most immunoassays target polysaccharides

**Advantages of proteins**
- Innumerable potential candidates
- Usually very potent antigens – easy to raise antibodies
- Can use bioinformatics for epitope prediction and assessment of “uniqueness”

**Disadvantages of proteins**
- Requires antibodies specific for two spatially independent epitopes – capture and indicator
- **Need for target discovery** – which of the potentially hundreds or thousands of expressed proteins are shed into body fluids in amounts sufficient for detection by immunoassay?

**Question** – Is it possible to take an unbiased look at target identification?
InMAD identifies only those antigens that are shed in vivo.
Additional strategies for target discovery

- Proteome arrays – probe arrays with InMAD immune serum
- Direct proteomic analysis
  - Collect serum or other body fluid from infected mouse or human
  - Identify microbial proteins
    - 1-D SDS-PAGE followed by LC-MS/MS
    - Multidimensional chromatography coupled to tandem mass spectrometry
- Once proteins are identified, assess uniqueness by query of databases
Target discovery for *Aspergillus fumigatus*

- Serum harvested from Aspergillus-infected BALB/c mice
- BALB/c mice immunized with serum from infected mice
- Immune serum collected
- Antigen prepared from *A. fumigatus*
  - Lysate
  - Culture filtrate
- Western blot probed with immune serum
  - Broad diffuse reactivity noted that is consistent with polysaccharide
  - Limited number of more discrete bands found that are consistent with proteins
  - Considerable mouse-to-mouse variability
Serum from an immunosuppressed and *A. fumigatus*-infected rabbit used to immunize a naïve rabbit

Detected predominantly a single antigenic moiety in mycelial extract

Detected antigen in serum and urine of infected rabbits

Detected antigen in leukemic child with IA

Subsequently identified as galactomannan
Advantages of antigen immunoassay
- Can identify antigens shed from distant site, e.g., IA
- Proven technology
- Easily adapted to point-of-care format
- Can be very inexpensive

Approaches to target discovery – varies with target category
- Polysaccharides – candidates often known in advance
  - Capsules are obvious targets, e.g., *C. neoformans*
  - Other polysaccharides such as LPS or galactomannan
- Proteins – rarely known in advance
  - Discovery complicated by very large number of potential targets
  - Target discovery by InMAD or alternative strategy such as proteomic analysis
  - InMAD has potential as a broad discovery platform