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Author(s): Aken Desai, Michael Mathis, 2008

Antibody-based Clinical Tests

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- Isotype: antigenic determinant on an immunoglobulin that is expressed by all members of a species
  - Inject a rabbit w/ a human immunoglobulin, determinants on constant region of the human antibody will be recognized as foreign
  - Rabbit will make antibodies against them
  - Rabbit antibodies recognize isotypic determinants and will react w/ Ig in the serum of virtually all humans
- Idiotype: antigenic determinant unique to a given Ig
  - Used to describe complementarity determining regions to a given antibody
  - Unique shape of epitope combining site made by the combination of the VH and VL hypervariable regions
- Many clinical tests use antibodies detect various proteins
  - Affinity is dissociation constant for the single interaction w/ single Fab (impractical, unmeasurable)
  - Avidity is interaction of multivalent antigen w/ multivalent antibody
- Immunoelectrophoresis
  - Separation of proteins by charge
  - Place serum from patient in one well, serum from normal individual in other well in middle of plate
  - Turn on electric field and separate charged proteins
  - Drop appropriate rabbit anti-human antisera from trough toward lane of fractionated sterum proteins
  - Antibodies in human serum detected as arcs of precipitation (where antigen=antibody)
- Agglutination
  - Combine antigen w/ known antibodies and look for reaction
  - A type blood w/ anti-A antibodies = reaction
  - B + anti-A antibodies = no reaction
- ELISA
  - Coat well w/ antibody anti-insulin epitope 1
  - Introduce sample, if insulin present, it will bind to antibodies
  - Wash off unbound material
  - Add anti-insulin-alkaline phosphatase epitope 2
  - If DNP changes from colorless --> yellow, insulin is present
- Radioimmunoassays
  - Use radiolabled antigens or antibodies as competitive assay
  - Clinical samples are used to compete for binding to antibodies w/ constant amount of radiolabeled standard
  - Standard curve helps determine amount bound
  - Results reported as positive/negative (50% bound is normal)
  - Quantitites based on standard curve
  - Titers (positive at dilution of 1:250, but not at 1:500)
    Amount of antibody against a particular pathogen should be zero or low in an individual who has never experienced that pathogen
  - After recovery, amount in serum should be higher
  - At height of infection, since not in effecter phase, antibody in serum should be low
    - Compare serum from patien tin active infection vs. convalescent serum
    - Pathogen-specific antibody titers elevated when pt. is first seen w/ subacute/chronic infection or one w/ long incubation period
- Monoclonal Antibodies
  - Mixture of serum antibodies in conventional (animal) antiserum is not perfect

        - Serum is mixture of antibodies that represents all antigens ever encountered
        - Each antigen w/ many epitopes results in the production of many antibodies in serum
        - Some antibodies bind to antigen w/ low affinity, may bind related antigen w/ low affinity
    - Monoclonal antibodies solve most of the problems inherent in conventional antisera
        - Spleen cells from mouse immunized w/ antigen A
        - Myeloma cells lacking antibody secretion and HGPRT (makes cell susceptible to death by aminopterin)
        - Spleen and myeloma cells fuse with PEG
        - Transferred to HAT medium w/ aminopterin and immortal hybridomas proliferate
        - Select hybridomas make antibody specific for antigen A
        - Clone selected hybridomas
    - Supernatant of each hybridoma can be screened via ELISA for secretion of particular antibody
    - Advantages: immoratal, monoclonal (specific), grown in large quantities
    - Uses: reagents for sandwich ELISA, detection of bacterial/viral antigens, tissue typing or analysis of CD expression, detection of virtually any protein