Histopathology of Fungal Infections

Diagnosis of Fungal Infections

• Diagnosis of a mycotic disease ideally includes:
  – Observation of typical symptoms
  – Demonstration of fungus in lesion with accompanying host reaction
  – Isolation of causative agent

• Not all the above can be accomplished in every type of fungal disease

• Other methods that can be used to aid in the diagnosis of a fungal infection include the detection of fungal:
  – Antigens
  – Antiboides
  – Metabolites
  – Cell wall markers

• More modern molecular-based methods are now available

• Some of the above methods are not yet available for many pathogenic fungi, particularly those that are somewhat unusual

• Remaining method for diagnosis includes the histopathological examination of biopsy material in order to observe:
  – Characteristic features of specific etiological agent
  – Host response to infection

Histological Stains for Fungi

• Hematoxylin and eosin (H&E)
  – Color of fungi: pink to pinkish blue
  – Applications:
    • Demonstrates inflammatory response
    • Stains some fungi
    • Allows determination of innate pigmentation by invading fungus
    • Demonstrates Splendore-Hoepli material
    • Stains most nuclei of yeast-like fungi
– Limitations:
  • Does not stain many fungi
  • Does not stain filamentous bacteria
  • Is not adequate for screening tissue with sparse number of fungal elements

• Gomori’s methenamine silver (GMS) [often referred to as ‘silver stain’]
  – Color of fungi: black brown on a light green background
  – Applications:
    • Stains most fungi, viable or not
    • Can stain filamentous bacteria
  – Limitations:
    • May overstain fungi and obscure internal details
    • Cannot detect host response

• Periodic acid-Schiff (PAS)
  – Color of fungi: red pink on a green background
  – Application: stains most fungi, viable or not
  – Limitations:
    • Masks innate color and internal details
    • Many tissue elements take up the stain
    • Cannot detect host response
    • Does not stain filamentous bacteria

• Gridley fungus (GF)
  – Color of fungi: purplish red on a yellow background
  – Application: stains most fungi
  – Limitations:
    • Masks innate color
    • Non-viable cells do not stain
    • Cannot demonstrate host response
    • Does not stain filamentous bacteria
• GMS with H&E counterstain
  – Stain of choice if only one slide available for histopathological examination
  – Color of fungi: black brown fungi on a red-pink background
  – Applications:
    • Permits study of host response
    • Excellent for detecting fungi and filamentous bacteria
  – Limitation: cannot determine innate fungal color

• Mucin (mucicarmine) stains
  – Mayer's or Southgate's preparations
  – Application: stains of mucopolysaccharide capsular material of fungi, e.g., Cryptococcus
  – Limitation: Not specific for Cryptococcus

• Modified Gram’s stains
  – Brown-Hopps’ and MacCallum-Goodpasture preparations
  – Application: stains Gram-positive filamentous bacteria
  – Limitation: does not selectively stain fungi

• Modified acid-fast stains
  – Ziehl-Neelsen’s and Kinyoun’s preparations
  – Application: stains Gram-positive filamentous bacteria
  – Limitation: does not stain fungi

• Modified Fontana-Masson
  – Applications:
    • Stains cell walls of Cryptococcus and other melanin producing fungi
    • Accentuates weakly pigmented agents of phaeohyphomycosis
  – Limitation: may stain fungal elements that are immature or innately not pigmented

• Whitening agents
  – Calcofluor White, Uvitex, and others
  – Application: stains cell walls of fungi
  – Limitation: need a fluorescent microscope
Histopathological Identification

- Tissue sections can be used to observe fungal elements and particular attributes that may be characteristic of certain species

- Fungi can appear as
  - Hyaline or pigmented (phaeoid)
  - One of four broad morphological categories
    - Yeast-like
    - Hyphae
    - Endosporulating spherules
    - Granules

- Other defining features of fungal forms in vivo include
  - Size and shape of cells
  - Cell wall thickness
  - Number and shape of blastoconidia (buds)
  - Presence or absence of septations
  - Capsules
  - Number of nuclei
  - Presence of pseudohyphae, hyphae, or arthroconidia

- Immunohistological staining is also used to detect and identify fungi in tissue
  - Can be direct or indirect staining, i.e., one step or multi-step process
  - Often fluorescent-tagged antibodies are used
  - Other ‘tags’ include
    - Gold-silver complexes
    - Enzyme complexes (e.g., peroxidases)