Viral Pathogens and Impostors: Who's Who in the Electron Microscope

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Introduction
Electron microscopy (EM) is invaluable in describing new viruses and in diagnosing viral illnesses. Ability to visualize whatever unknown organism may be present in a sample is advantageous, compared to searching for a mysterious agent using a biochemical test with specific markers. All molecular tests that identify a given virus require the use of a probe, i.e., antibodies with secondary visual markers, polymerase chain reaction (PCR), and polyacrylamide gels of nucleic acids and proteins all require reagents (specific antibody, PCR probes, and nucleic acid or protein standards). Selecting the correct reagent when the pathogen is unknown can be time consuming and expensive, and in some cases, no reagents are available. Additionally, mutant genomes may not react in PCRs, and there can be antibody cross-reactions or no reaction with immunological reagents.

EM has further advantage in that it does not require live viruses as does cell culture. Viruses may die during transport, and some cannot be grown in routine tissue culture systems. Finally, EM is relatively fast (0.5-1 hr for viruses in fluids, and 3-24 hr for infected tissues).

Discussion
Electron microscopy is an invaluable adjunct in viral diagnosis if viral concentration in fluids is high enough (10^5-10^6 particles/ml) and in tissues if viral infection is not too small or focal. Procedures, results, and interpretations are listed in outline form below.

Methods

Viral specimen preparation and examination techniques
Negative staining for viruses in fluids
0.5 – 2 % aqueous uranyl acetate, ammonium molybdate, or phosphotungstate
Examine fluids at 40,000-60,000x for 20-30 min.
Thin sectioning for viruses in tissue
Any routine EM tissue preparation procedure that includes aldehyde fixation, osmication, possibly uranyl acetate en bloc staining (not required), dehydration, and embedment in epoxy resin.
Examine tissues between 20,000-40,000x.

Problems and pitfalls
EM is less sensitive than PCR; viruses may be too dilute in fluids.
Focal infections can be missed in tissues.
Look-a-likes and masqueraders can mimic viruses.
Bacteria and other organisms can produce confusing structures. Organisms “on” or ‘in’ sections may resemble or obscure viruses (if “on” sections, they are contamination in reagents/washes; if “in” sections, they could be contamination or actual pathogens).

Bacteriophages in water may look like pathogens. Source of the sample should be considered (An endoscope can carry down oral flora; stored specimens and water sources can harbor bacteria that can produce bacteriophages. Suboptimal specimens (Viruses may or may not be recognizable.)

Frozen
Wax embedded
Stained section on a glass slide
Dried on side of container
Formalin fixed
Immuno fixed (Michel’s fixative)
Unfixed and water- or buffer-stored

Ways to get around problems
Ultracentrifugation to concentrate viruses in fluids
Antibody concentration (Kapikian & Dienstag)
Confocal microscopy of tissue slabs to select areas of pathology (Miller et al., 1997)
Sampling multiple tissue locations
If the only tissue available is in a paraffin block: de-paraffinize in xylene, stain with Os, and embed.
If all the tissue has been sectioned and stained on a glass slide, embed in situ, and glue slab onto a blank block.
If fixed in light microscopy fixative, transfer to glutaraldehyde.
Where to look in tissues: inflammation, nucleated cells, necrosis edge (not center), unusual ultrastructure for the tissue type
Thaw frozen specimens in glutaraldehyde.

Results

Morphological types of human viruses
Naked (non-enveloped) icosahedral (spherical like a baseball)
RNA or DNA
3 size ranges
Large (70-90 nm, e.g., adenovirus, rotavirus)
Medium (40-55 nm, e.g., polyomavirus, papillomavirus)
Small (20-35 nm, e.g., parvovirus, picornavirus, calicivirus)
“Capsid” or “nucleocapsid” in this case is the same thing as the complete “virion”
Enveloped (nucleocapsid surrounded by protein membrane; usually pleomorphic like a raisin or an underinflated beach ball)
RNA or DNA
Nucleocapsids
Icosahedral (20-120 nm, e.g., herpesvirus)
Filamentous 18 x ≤1500 nm, e.g., paramyxovirus
Complex (dumbbell-shaped with lateral bodies, poxvirus)

Complete virions (nucleocapsid surrounded by envelope) (40-400 nm)
Small (40-70 nm, e.g., flavivirus, alphavirus)
Medium (100-150 nm, e.g., coronavirus, orthomyxovirus, bunyavirus)
Large (100-400 nm, e.g., paramyxovirus, poxvirus)
Globular (e.g., paramyxovirus)
Filamentous (e.g., Ebola virus)
Brick-shaped (e.g., vaccinia a poxvirus)

**Things that can look like viruses but are not**

**In negative stains of fluid samples**
Resemble spherical virions or nucleocapsids
   - Lipid droplets
   - Protein droplets
   - Membrane vesicles
   - Ribosomes
   - Tailless Bacteriophages (are viruses, but non pathogenic)
Resemble filamentous nucleocapsids
   - Crystalline structures such as salt precipitates
   - Flagella and pili; fibers from clothing, gauze, food, and filters
   - Strands or liquid boundaries in sputum and urine
Resemble spiked enveloped viruses
   - Inside-out mitochondrial membranes
Resemble poxviruses
   - Melanosomes
Resemble viral capsid material
   - Bacteriophage neck and tail proteins
   - Bacterial cell wall debris

**In thin sections of infected tissue**
Resemble spherical virions or nucleocapsids
   - Ribosomes
   - Microtubules cut in cross section (look like parvovirus)
   - Glycogen
   - Clathrin coated vesicles
   - Membrane vesicles (caveolae, pinocytotic vesicles, lysosomes, phagosomes, peroxisomes, Golgi vesicles, synaptic vesicles, microvesicles, cell debris from lysed cells)
   - Neurosecretory vesicles
   - Nuclear granules
   - Nuclear bodies
   - Nuclear pores
   - Mitochondrial dense granules
   - Microvilli and collagen in cross section
Resemble filamentous nucleocapsids
   - Microtubules
Intermediate filaments
Amyloid
Amyloid
Cryoglobulin
Tubuloreticular inclusions (TRI) (seen in lupus, HIV infection, interferon production or therapy
Clumped filamentous chromatin
Resemble spiked enveloped viruses
Clathrin-coated vesicles
Resemble poxvirus
Melanosomes

Conclusions
Electron microscopy is frequently used for identifying viruses because it does not require an a priori notion of which agent might be present for reagent selection and because it can yield rapid results. However, in negative stains of liquid specimens, artifacts and cell debris may resemble viruses. Also, in thin sections, normal cell organelles and/or artifacts resulting from non-viral causes, including delayed or improper fixation, can be confusing. Knowledge of proper specimen preparation methods, virus ultrastructure, potential confusing elements, and normal cell organelles is essential to reliable virus identification.

References and Resources
Kapikian AZ, Dienstag JL, Purcell RH. Immune electron microscopy as a method for the detection, identification, and characterization of agents not cultivable in an in vitro


Online:
The Big Picture Book of Viruses
http://www.virology.net/big_virology/

All the Viruses on the WWW
http://www.virology.net/garryfavweb11.html

Animal Viruses with Emphasis on Pathogens of Humans
http://textbookofbacteriology.net/themicrobialworld/AnimalViruses.html