Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Clinical Immunology Newsletter

Vol. 3, No. 7 April 7, 1982

Immunoelectron Microscopy in Diagnostic Virology

Max A. Chernesky, Ph.D.
Regional Virology Laboratory
Department of Pediatrics
McMaster University
Hamilton, Ontario, Canada

Immunoelectron microscopy (IEM) allows the visualization of antigen-antibody interactions in an electron microscope (EM). The technique was first described in 1941 (9, 10), using plant viruses and their antibodies, and has been employed in many biologic systems. Since that time, a series of procedures for IEM have appeared. Several excellent reviews have been published on: a) the morphology of antibody and virus-antibody interaction (7, 25); b) electron and immunoelectron microscopic procedures for diagnosis of viral infections (18); c) practical aspects of the IEM of intracellular structures (34, 45); d) the role of EM in diagnostic virology (16); e) IEM as a method for the detection, identification and characterization of agents not cultivable in an in-vitro system (31). This article will review the various methods of IEM and illustrate their usefulness in diagnostic virology.

The clearness of the end reaction will depend upon several factors, including the purity of the antibody and antigen preparations. Antisera should be heat-inactivated to destroy complement, which could induce virolysis in some systems (7). IEM methods may be grouped into liquid or solid phase approaches. Liquid phase techniques include steps to concentrate the immune complexes for easier viewing, whereas solid phase techniques avoid these extra steps. Concentration is usually performed by centrifugation, chromatography or diffusion of the liquid phase through agar.

Liquid Phase Techniques

Direct Method

This has been the most commonly used IEM method for viewing virus immune complexes prepared from tissue culture isolates or clinical specimens. The recommendation of Almeida and Waterson (7) involves the interaction of equal volumes of concentrated antigen with dilutions of antiserum (undiluted, 1:10, 100) at 37°C for 1 hour; the solution is then left overnight at 4°C. The immune complexes are centrifuged the following morning. The pellet is resuspended in a small volume of distilled water, then stained with phosphotungstic acid (PTA) on a grid before viewing.

Although the EM is being used near the limit of practical resolution in these studies, basic phenomena concerning antibody structure and function have been reported. Using ferritin (20) or dinitrophenyl-polymethylenediamine (DNP) (49) as antigens, the dimer structure of immunoglobulin G (IgG) was confirmed with the two Fab portions in the form of a "V" and the Fc fraction forming the leg of a "Y" shaped molecule. Treatment with pepsin digested the Fc portion (49), and papain hydrolysis produced small rod-like structures 60-80 Å long. Using polyomavirus as antigen, Almeida et al. (3) digested IgG antibody with papain and observed rigid, radially oriented spikes approximately 70-90 Å surrounding the virus particles, instead of the normal halo of flexible, randomly oriented molecules of 250 Å length in untreated serum specimens. There are many studies describing the morphology of IgM, but few deal with conformational changes during binding (25). IgM molecules have been visualized in the EM by employing erythrocyte membrane fragments (7), bacterial flagella (21) or foot and mouth disease virus (2) as antigens. They appear as long (~350 Å), flexible, looping molecules in the shape of a five-pointed star with 10 proposed binding sites (7). Using a peroxidase IEM technique, immunoglobulins have been detected inside the rough endoplasmic reticulum, perinuclear

In This Issue

space, and Golgi apparatus of human lymphoid cells from a variety of sources (39).

Direct IEM methodology has been used to study basic morphology of several viruses that present a problem with ordinary conventional EM. Antibody attachment to rubella virus allows its separation from contaminating debris of equal size and density and a morphological definition of the periphery of the virus particles (13). Similarly, IEM has enabled basic observations on the morphology of rhinoviruses (30) and polyoma and wart viruses (4). IEM has also contributed to our knowledge of diseases caused by viruses that have been difficult or impossible to cultivate in vitro. During an outbreak of nonbacterial gastroenteritis (NBG) in Norwalk, Ohio in 1971, Kapikian and coworkers reacted stool filtrate with convalescent serum from a diseased volunteer and observed 27-nm particles in aggregates surrounded by clumped antibodies (28). Subsequently, it was found that certain individuals with NBG develop serologic evidence of infection detectable by IEM. IEM methods were also used to demonstrate experimental infection (46), and further outbreaks of human disease were identified by related agents from Hawaii and Maryland (47). Several other agents resembling the Norwalk virus in morphology have been identified, and their relationship to the Norwalk group has been studied by IEM. Brandt and coworkers (14) have recently compared direct EM, IEM, and enzyme immunoassay for the detection of viruses in feces and rectal swabs. Used with commercial gamma globulin, IEM had a distinct advantage with all viruses detected in rectal swabs and small 27-nm particles in feces, but added little to increase the number of rotaviruses or adenoviruses viewed in stool. The relationship between hepatitis A virus (27-nm particles) and infectious, non-B viral hepatitis was uncovered using similar IEM techniques (31). Coproantibodies A and M to hepatitis A virus, as well as the virus itself, have been detected in the feces of a small percentage of patients within 10 days of clinical hepatitis (36). The morphologies of hepatitis B virus and its markers (5) and rotavirus (22) have been studied using similar methods. Coronaviruses (29) have been rescued from cell or organ cultures and viewed under the EM by forming immune complexes with serum from patients convalescing from respiratory disease. The direct IEM technique has also been used for serotyping within major virus groups, including the papovaviruses (23, 42), picornaviruses (8, 15, 27, 43) and adenoviruses (50, 51).

Almeida et al. (1) recently introduced a direct IEM method that adds prepared virus to EM-negative material before the addition of virus-specific antiserum. Mixed immune aggregates (MIA) of rotavirus and of hepatitis B surface antigen (HBsAg) were observed when tested on a low molecular weight subunit of rotavirus and a micellar form of HBsAg, respectively. Thus, these MIAs demonstrated that the unknown components had antigens in common with the established virus or antigen.

Although IEM direct methodology had been used extensively for the serologic diagnosis of viral diseases such as hepatitis A and Norwalk virus gastroenteritis, it has, for technical reasons, been replaced by hemagglutination and radio- or enzyme- immunoassays (31).

Indirect Method

Using a technique similar to methods for fluorescent antibody studies, some workers have employed a second antispecies antibody, with or without a label, to enhance the appearance of virus-antibody complexes formed in the direct IEM method. The procedure as described for adenovirus identification (50) from cell culture harvest or clinical specimens involves the incubation of an optimal concentration of rabbit antihuman IgG and the adenovirus immune complexes in equal volumes at 6 °C overnight. Using these procedures, these workers demonstrated enhanced viewing of virus particles and greater sensitivity than by the direct method.

Ferritin-labelled antispecies antibodies have been used in the indirect method for the demonstration of antibody attachment to influenzavirus (41) and hepatitis B core antigen (26). A rapid indirect noncentrifugation ferritin method for the identification of rotaviruses, adenoviruses, and Coxsackie virus B-5 has recently been described (12).

Agar Gel Diffusion (AGD)

Kelen et al. (33) first described a modification of the direct IEM technique using agar gel diffusion (AGD) filtration to concentrate immune complexes of HBsAg. After incubating virus and antiserum at 37 °C for 30 minutes, they deposited microdrops on a 0.8% agar surface. A formvar-carbon-coated grid was floated on top of the last drop and removed just as the last part of the fluid phase disappeared into the agar.

Employing mouse ascitic fluids against alphavirus, flavivirus, bunyavirus and rhabdovirus antigens with homologous and heterologous antibodies, Fauvel et al. observed specific clumping by AGD-IEM; IEM antibody titers were comparable to hemagglutination inhibition (19).

Serum in Agar (SIA) Method

Anderson and Doane described this modification of the AGD technique and used it to serotype enteroviruses by incorporating the antisera into the agar (8). Dilutions of single or pooled reference sera were added to a cool molten solution of 1% agar in microtiter plate wells. Formvar-carbon-coated grids were placed on the surface of the agar, and the viral specimens (1 or 2 microdrops) were added and allowed to dry. Homologous antibodies rapidly diffuse from the agar to the specimen, and immune complexes are easily seen in the EM after negative staining. These authors

have reported the SIA-IEM method to be as sensitive as the direct method and have used it to type other viral groups.

Berthiaume et al., using commercially available pools of human gamma globulins, instead of virus-specific antisera, detected several gastroenteric viruses in clarified feces and cell culture supernatant fluids (11). Rotaviruses, adenoviruses, astroviruses, picornaviruses, parvoviruses, and coronaviruses were detected. In this study the specimen was dropped onto the agar surface and then picked up on an inverted grid similar to the AGD method. Using these techniques, Trepanier et al. made a comparison of direct EM with direct gamma globulin SIA and indirect ferritin IEM techniques on feces samples submitted to a diagnostic laboratory (48). These two approaches enumerated 25 and 103 more rotaviruses per grid square, respectively, as compared to the direct EM technique. Similarly, Lamontagne and coworkers (35) found specific IEM 100 times more sensitive for detecting rotaviruses and adenoviruses and up to 10 times more sensitive for bovine herpes virus.

Solid Phase Techniques

Solid phase immunoelectron microscopy (SPIEM) was first described in 1973 by Derrick, who coated grids with capture antibody (CA) to plant viruses (17). Milne and Luisoni (37) shortened and simplified the technique and incorporated a decorator antibody (DA) into the method. Shukla and Gough (44) enhanced the adsorption of plant virus antibodies to the grid by using staphylococcal protein A (SPA) as a binding agent. This method, with the addition of a DA, increased the sensitivity of detecting viruses in plant extracts from 7 to 68-fold. This technique without DA has been reported for the detection of rotavirus; it was 3.5 times more sensitive than direct EM of 25% feces suspensions (40). The following procedure was used by Giraldo et al. (24) for the rapid detection of papovaviruses: with the use of locking EM forceps, a drop of optimally diluted CA was placed on the grid for 5 min and then washed with phosphate-buffered saline. A drop of the virus suspension was added and washed after a 15-min incubation. DA was incubated on the grid for an additional 15 min. The final washed preparation was stained with 1% uranyl acetate for 2 min before draining and drying. With this method, a 28-fold increase of virus particles over noncoated grids was observed, and virus preparations at concentrations of 10^2-10^3 PFU/ml could be detected. DA facilitated easier viewing, and SPA allowed the use of lower-titered CA.

Whole *Staphylococcus aureus* organisms have been used as the solid phase in a SPIEM method (32). Rabbit anti-Sindbis virus serum was mixed with the bacteria, and the resulting pellet was washed and used to extract virus from tissue culture supernatant fluid, with the use of centrifugation to concentrate. The preparation was stained with phosphotungstic acid on a grid for transmission EM, and polylysine-coated cover glasses were used for scanning EM. This technique, similar to the other SPIEM methods, avoids a prozone effect (see next section) and specific migration on the grid of virus particles (6). Sindbis virus particles were seen attached to the surfaces of the bacteria.

A SPIEM technique has been described for measuring antibodies (38). This antigen-controlled immunodiagnosis test coated grids with bacteriophage T4, vaccinia virus, or *Yersinia enterocolitica* bacteria with trapped homologous antibodies that were subsequently highlighted under the EM by the addition of SPA-coated gold sol that was prepared previously by ultrasonication.

Comments on Interpretation

In most cases, it is sufficient to use the presence of complexes to signify a positive response. In liquid phase techniques, the concentration of antibody in relation to the amount of virus in the preparation will markedly affect the appearances of the complexes. At low antibody concentrations, the aggregates will be very small, consisting of a few clearly outlined virus particles. As antibody concentrations increase, the aggregates will become larger and more numerous; antibody layers will become thicker, and the virion surface details will appear less precise. With antibody excess, individual virions will be found surrounded by an antibody halo, the number of aggregates will be greatly reduced, and virus particles may be difficult to recognize (prozone effect).

Conclusions

Advances in the use of IEM as a diagnostic procedure have been relatively slow since the first description of the technique in 1941 by Anderson and Stanley, using tobacco mosaic virus (9). The reasons for its slow development include the need for both a complex and expensive microscope and highly trained personnel with particular interests. During the ensuing 40 years, IEM has been used to visualize antibody molecules and various interactions between antibodies and antigens, especially associated with microorganisms.

References

1. **Almeida, J. D., et al.** 1981. The use of markers in immune electron microscopy. J. Virol. Meth. 2:169-174.
2. **Almeida, J. D., F. Brown, and A. P. Waterson.** 1967. The morphologic characteristics of 19S antibody. J. Immunol. 98:186-193.
3. **Almeida, J. D., B. Cinader, and D. Naylor.** 1965. Univalent fragments of antibody: A study by electron microscopy. Immunochemistry 2:169-176.
4. **Almeida, J. D., and A. P. Goffe.** 1965. Antibody to wart virus in human sera demonstrated by immune electron microscopy and precipitin tests. Lancet ii:1205-1207.
5. **Almeida, J. D., D. Rubenstein, and E. J. Stott.** 1971. New antigen-antibody system in Australia-antigen-positive hepatitis. Lancet ii:1225-1227.

6. **Almeida, J. D., L. M. Stannard, and A. S. M. Shersby.** 1980. A new phenomenon (SMOG) associated with solid phase immune electron microscopy. J. Virol. Meth. 1:325-330.
7. **Almeida, J. D., and A. P. Waterson.** 1969. The morphology of virus-antibody interaction. Adv. Virus Res. 15:307-338.
8. **Anderson, N., and F. W. Doane.** 1973. Specific identification of enteroviruses by immune electron microscopy using a serum-in-agar diffusion method. Can. J. Microbiol. 19:585-589.
9. **Anderson, T. F., and W. M. Stanley.** 1941. A study by means of the electron microscope of the reaction between tobacco mosaic virus and its antiserum. J. Biol. Chem. 139:339-344.
10. **Ardenne, M. von, H. Friedrich-Freksa, and G. Schramm.** 1941. Electronenmikroskopischen untersuchung der precipit inreaktion von tabakmosaikvirus mit kaninchenantiserum. Arch. Gesamte Virusforsch. 2:80-86.
11. **Berthiaume, L., et al.** 1981. Rapid detection of human viruses in faeces by a simple and routine immune electron microscopy technique. J. Gen. Virol. 55:223-227.
12. **Berthiaume, L., et al.** 1981. Rapid identification of viruses by a simple indirect immune electron microscopy technique using ferritin-labelled antibodies. J. Virol. Meth. 2:367-373.
13. **Best, J. M., et al.** 1967. Morphological characteristics of rubella virus. Lancet ii:237-239.
14. **Brandt, C. D., et al.** 1981. Comparison of direct electron microscopy, immune electron microscopy, and rotavirus enzyme-linked immunosorbent assay for detection of gastroenteritis viruses in children. J. Clin. Microbiol. 13:976-981.
15. **Chaudary, R. K., D. A. Kennedy, and J. C. N. Westwood.** 1971. Serological cross reactivity within the picornaviruses as studied by electronmicroscopy. Can. J. Microbiol. 17:477-480.
16. **Chernesky, M. A.** 1979. The role of electron microscopy in diagnostic virology, pp. 125-142. *In* D. Lennette, et al. (eds.), Diagnosis of viral infections: The role of the clinical laboratory. University Park Press, Baltimore.
17. **Derrick, K. S.** 1973. Quantitative assay for plant viruses using serologically specific electron microscopy. Virology 56:652-653.
18. **Doane, F. W., and N. Anderson.** 1977. Electron and immune electron microscopic procedures for diagnosis of viral infections, pp. 505-539. *In* E. Kurstak and C. Kurstak (eds.), Comparative diagnosis of viral diseases II. Academic Press, New York.
19. **Fauvel, M., H. Artsob, and L. Spence.** 1977. Immune electron microscopy of arboviruses. Amer. J. Trop. Med. Hyg. 26:798-807.
20. **Feinstein, A., and A. J. Rowe.** 1965. Molecular mechanism of formation of antigen-antibody complex. Nature 205:147-149.
21. **Feinstein, A., and E. A. Munn.** 1966. An electron microscopic study of the interaction of macroglobulin (IgM) antibodies with bacterial flagella and of the binding of complement. J. Physiol. (Lond.) 186:64P-66P.
22. **Flewett, T. H., et al.** 1974. Relation between viruses from acute gastroenteritis of children and newborn calves. Lancet ii:61-63.
23. **Gardner, S. D., et al.** 1971. New human papovavirus (B.K.) isolated from urine after renal transplantation. Lancet i:1253-1257.
24. **Giraldo, G., et al.** 1982. Solid-phase immune electron microscopy double antibody technique (SPIEM-DAT) for the rapid detection of papovaviruses. J. Clin. Microbiol. 15:38-42.
25. **Green, N. M.** 1969. Electron microscopy of the immunoglobulins. Adv. Immunol. 11:1-30.
26. **Huang, S. N., and A. R. Neurath.** 1979. Immunohistologic demonstration of hepatitis B viral antigen in liver with reference to its significance in liver injury. Lab. Invest. 40:1-17.
27. **Hummeler, K., T. F. Anderson, and R. A. Brown.** 1961. Identification of poliovirus particles of different antigenicity by specific agglutination as seen in the electron microscope. Virology 16:84-90.
28. **Kapikian, A. Z., et al.** 1972. Visualization by immune electron microscopy of a 27-nm particle associated with acute infectious nonbacterial gastroenteritis. J. Virol. 10:1075-1081.
29. **Kapikian, A. Z., et al.** 1973. Detection of coronavirus strain 692 by immune electronmicroscopy. Infect. Immun. 7:111-116.
30. **Kapikian, A. Z., J. D. Almeida, and E. J. Stott.** 1972. Immune electron microscopy of rhinoviruses. J. Virol. 10:142-146.
31. **Kapikian, A. Z., J. L. Dienstag, and R. H. Purcell.** 1980. Immune electron microscopy as a method for the detection, identification and characterization of agents not cultivable in an in vitro system, pp. 70-83. *In* N. R. Rose and H. Friedman (eds.), Manual of clinical immunology, 2nd ed. American Society for Microbiology, Washington, D.C.
32. **Katz, D., et al.** 1980. Solid phase immune electron microscopy (SPIEM) for rapid viral diagnosis. J. Immunol. Methods 38:171-174.
33. **Kelen, A. E., A. E. Hathaway, and D. A. McLeod.** 1971. Rapid detection of Australia/SH antigen and antibody by a simple and sensitive technique of immunoelectron-microscopy. Can. J. Microbiol. 17:993-1000.
34. **Lake, J. A.** 1979. Practical aspects of immune electron microscopy. Methods Enzymol. 61:250-257.
35. **Lamontagne, L., et al.** 1980. Diagnosis of rotavirus, adenovirus, and herpesvirus infections by immune electron microscopy using a serum-in-agar diffusion method. Can. J. Microbiol. 26:261-264.
36. **Locarnini, S. A., et al.** 1980. Coproantibodies in hepatitis A: Detection by enzyme-linked immunosorbent assay and immune electron microscopy. J. Clin. Microbiol. 11:710-716.
37. **Milne, R. G., and E. Luisoni.** 1975. Rapid high-resolution immune electron microscopy of plant viruses. Virology 68:270-274.
38. **Muller, G., and C. L. Baigent.** 1980. Antigen controlled immunodiagnosis—ACID test. J. Immunol. Methods 37:185-190.
39. **Newell, D. G., et al.** 1980. The intracellular localisation of immunoglobulin in human lymphoid cells and haematopoietic cell lines by immunoperoxidase electron microscopy. J. Immunol. Methods 37:275-286.
40. **Nicolaieff, A., G. Obert, and M. H. V. Van Regenmortel.** 1980. Detection of rotavirus by serological trapping on antibody coated electron microscope grids. J. Clin. Microbiol. 12:101-104.
41. **Patterson, S.** 1975. Detection of antibody in virus-antibody complexes by immunoferritin labelling and subsequent negative staining. J. Immunol. Methods 9:115-122.
42. **Penney, J. B., and O. Narayan.** 1973. Studies of the antigenic relationship of the new human papovaviruses by electron microscopy agglutination. Infect. Immun. 8:299-300.
43. **Petrovicova, A., and A. S. Juck.** 1977. Serotyping of Coxsackieviruses by immune electron microscopy. Acta virol. (Praha) 21:165-167.
44. **Shukla, D. D., and K. H. Gough.** 1979. The use of protein A from *Staphylococcus aureus* in immune