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Infectious Mononucleosis: Observations on Transmission

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Epstein-Barr virus oropharyngeal shedding has been demonstrated in infectious mononucleosis patients many months after acute illness and long after the disease hallmarks, atypical lymphocytes and heterophile antibody, have disappeared. Extracellular virus is present more frequently in saliva than in other oropharyngeal samples. Prolonged excretion of EBV in asymptomatic carriers explains the difficulty in tracing case-to-case spread and increased transmissibility in age groups in which salivary exchange is high.

Epidemiological thinking about infectious mononucleosis (IM) has undergone extensive revision in a relatively short period of time. Since 1968 when Epstein-Barr virus (EBV) antibody responses during the disease were first demonstrated, a surge of information has established the etiologic role of EBV [1,2]. Data supporting the causal relationship have been derived from multiple lines of investigation utilizing serologic, virologic, and immunologic techniques: (1) EBV antibodies measured by immunofluorescence, complement fixation, and neutralization are regularly absent before infectious mononucleosis, appear during the course of illness, and persist for years thereafter; (2) specific antibodies of the immunoglobulin M type are detectable early in illness, indicating a primary infection; (3) EBV can be recovered from leukocyte cultures of IM patients; (4) the presence or absence of EBV antibodies correlates with resistance or susceptibility to infectious mononucleosis; (5) the classic heterophile antibody of IM develops in squirrel monkeys inoculated with EBV-transformed leukocytes; and (6) oropharyngeal excretion of EBV has been demonstrated in infectious mononucleosis patients.

Elucidation of the behavior of IM in different environmental and sociologic conditions has evolved to a large extent through sero-epidemiologic studies of EBV antibody. Such investigations have shown that the virus is a common and widely disseminated agent throughout the world and that the majority of EBV infections are subclinical and inapparent. Infectious mononucleosis is a well-recognized disorder in populations with advanced sociohygienic standards; here, the peak incidence of disease occurs in adolescents and young adults. Conversely, the disease is a rarity in crowded, densely populated, developing regions with lower standards of hygiene.

Because antibody to the viral capsid antigen (VCA) of EBV persists for many years, probably for life, measurements of this IgG antibody in any age group define cumulative prevalence rates of all persons having previous or current infections and

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associated immunity [3,4]. For example, investigations utilizing this marker have demonstrated extremely early acquisition of primary EBV infection in African and Melanesian populations. Biggar et al. studying newborn children in Accra, Ghana, found that by the age of 18 months 82 percent had been infected [5]. In three remote, genetically distinct Melanesian populations 89 percent of two-year-old infants were seropositive for EBV. Lang et al. describe the intense interpersonal contact and primitive personal hygiene of these social groups which provide an optimum setting for early acquisition and spread of EBV infection [6].

In contrast, Henle and Henle found that, in the Cleveland Family Study, representing closed western society nuclear groups, the majority of children had escaped EBV infection up to the age of ten years. Evidence of spread was found in approximately one-third of family groups, but transmission from parents to children was not a regular event. Likewise, spread of the virus among siblings was infrequent, neither from older to younger children, nor vice versa [7]. Joncas and Mitnyan followed 67 EBV-antibody negative members of 75 Canadian families over a period of approximately two years; only 10.5 percent of these susceptibles developed asymptomatic seroconversion [8].

Investigations of college and military populations have repeatedly demonstrated the low contagiousness of infectious mononucleosis to susceptible and exposed roommates of index IM cases [3,4,9,10]. Prospective studies of university students in the United States and Great Britain indicate EBV infection rates of only 12–13 percent per year among susceptibles. In recent work at Yale University, 18 antibody-negative roommates or close contacts of 17 IM patients were observed over a period of nine months [11]. In this group only one contact developed clinical illness associated with elevated heterophile antibody 41 days after exposure. No clinical symptoms of mononucleosis or evidence of EBV seroconversion occurred in the other 17 subjects. The secondary attack rate of one of 18 roommates (5.5 percent) was lower than the overall university EBV infection rate of 13.1 percent.

In studies of other age groups, sera from six-year-old school children entering grammar schools located in upper and lower socioeconomic districts in New Haven, Connecticut, were tested for EBV antibody prevalence and compared to matched samples collected four years later. The EBV seroconversion rate was 50 percent in children from less economically privileged areas and only 2.4 percent in the more advantaged groups. Taken together, such serologic results, demonstrating early acquisition of subclinical EBV infection in infants from developing countries, in children from lower socioeconomic sectors of developed regions, and in nurseries and institutions, indicate that close personal contact is a common denominator for circulation of EB virus in young age groups. These findings contrast with the low contagiousness of adults with clinical infectious mononucleosis and scant evidence that the disorder occurs in epidemic form.

Unlike some contact diseases, such as measles and rubella, in which the disease passes from one recognized case to another, it has been difficult to trace transmission of infectious mononucleosis on a case-to-case basis. Numerous efforts, undertaken as early as 1937, to identify the etiology and to transmit IM were made by many investigators. In such attempts utilizing different routes of administration, 91 volunteers received inocula including blood, serum, throat and nasal washings, lymph node suspensions, and stool suspensions obtained from acutely ill patients. The results were unsuccessful and inconclusive except for one trial, reported by Wising in 1942. In this investigation the disease was transmitted to a 23-year-old female volunteer who received 250 ml of heparinized whole blood obtained from a 31-year-

old donor during the first week of clinical infectious mononucleosis. Approximately three weeks following transfusion, the recipient developed full-blown clinical disease together with heterophile antibody production [12].

Identification of the etiologic role of Epstein-Barr virus promptly opened the way for studies of factors involved in transmission of IM. The role of the oropharynx as a dominant route of transmission had been suggested earlier by Hoagland's observations which raised the possibility of actively infectious saliva; these studies also indicated the existence of a long incubation period of 34–49 days [13]. An essential step for investigations of such EBV transmission was the development of a sensitive marker for detection of virus in oropharyngeal secretions. The assay was based on the unique property of EBV to transform or immortalize primary cultures of fetal leukocytes. Proof that the transforming agent, detected in throat washings from IM patients, was EBV included (1) virus-specific complement fixation antigens appeared in transformed fetal leukocytes, and (2) the transforming activity of the throat washings was neutralized by antibody to EBV. Furthermore, when the same throat washings, capable of inducing only complement fixation antigen in umbilical cord leukocytes, were added to adult human or marmoset leukocytes, the transformants contained both immune fluorescent and complement-fixing antigens [14,15,16]. Thus, EBV expressions differed in the transformants of different types of cells.

Electron microscopic studies of concentrated throat washings helped to elucidate the physical nature of the leukocyte-transforming factor [17,18]. Enveloped herpes virus was observed in concentrated, throat-washing material, obtained from an EBV antibody-positive patient receiving immunosuppressive therapy for prevention of renal transplant rejection. On examination, ten virions were observed in a small drop of the final preparation and the concentrated specimen contained approximately 3×10^8 virions/ml. Transforming activity of the throat washing with viral particles was neutralized only by EBV antibody-positive sera, and no other human herpes viruses were present in the specimen. The findings provided direct evidence that the leukocyte-transforming factor in oropharyngeal specimens is EBV, and clearly supported the concept that mature enveloped EBV is released by some cell in the oropharynx.

Other investigations have approached questions regarding viral persistence and sites of oropharyngeal production in several ways: (1) a group of IM patients was studied serially to determine whether oropharyngeal excretion was continuous or intermittent, (2) throat washings were examined by procedures to determine whether virus excretion was "cell-associated" or "cell-free," and (3) specimens were collected from multiple oropharyngeal sites in attempts to localize the sites of virus production.

Three general patterns of EBV oropharyngeal excretion were observed in studies of 104 serial throat washings collected from 20 IM cases. Three (15 percent) patients shed virus continuously, beginning in the second week through the third month after onset. The majority (75 percent) excreted virus intermittently throughout the first three months and as long as 18 months after acute illness. Conversely, serial throat washings from two other IM patients lacked detectable virus in all specimens. Techniques eliminating infected cells in throat washings did not alter their capacity for transformation and indicated that EBV is located extracellularly in the oropharynx. A small amount of virus was commonly present in saliva and also detected in fluids collected by swabbing multiple sites of the oropharynx, including the orifices of Stensen's ducts. Such findings implicated the salivary glands as one potential site of EBV production and suggested that virus is released by a cell in

salivary glands, perhaps ductal or glandular epithelium or by specialized lymphoid cells [19]. Demonstration of the presence of EBV-DNA in exfoliated buccal mucosa cells by in situ nucleic acid hybridization techniques suggests that epithelial cells of the buccal mucosa may also be a source of EBV [20].

In other investigations of viral shedding, cells and cell-free parotid secretions, saliva, and throat washings from young adult patients with acute IM and patients of similar age undergoing tonsillectomy were assayed for the presence of infectious EB virus. From 21 patients undergoing tonsillectomy, secretions were obtained by cannulation of the parotid ducts; in a group of 19 IM patients, saliva was aspirated from the orifice of Stensen's duct. Seventeen of 21 patients from whom samples were obtained by cannulation were seropositive for EBV; infectious EBV was found in specimens from two of these subjects. In one tonsillectomy case who had IM two and a half months previously, saliva from both parotid ducts contained EBV; the virus was also present in saliva cannulated from the right parotid gland of another seropositive patient without a history of IM. Oral saliva from both subjects contained EBV; in one case, more virus was present in the cannulated specimen than in the oral sample. EBV was not recovered from extracts of tonsil lymphocytes of eight subjects, including both individuals whose parotid ducts yielded virus. Saliva aspirated from the orifice of Stensen's duct contained EBV in eight of 19 patients with acute IM, and all had detectable virus in oral salivary specimens [21].

Taken together, these findings indicate that the salivary glands are a major site of oropharyngeal production of Epstein-Barr virus and shed light on transmission of the infection. Such low-level, long-term EBV production in salivary glands may stimulate life-long maintenance of viral capsid antigen, membrane antigen, and specific nuclear antigens. From a theoretical standpoint, it is also possible that primary EBV replication takes place in the salivary gland before B lymphocytes are infected and some individuals may have only salivary gland infection in the absence of leukoviremia.

Person-to-person transmission of viral infection is influenced by both the pattern and sites of excretion of the agent. To a large extent, contagiousness correlates with the titers of virus excreted; patients who shed large amounts of virus are likely to be highly infectious. For example, in diseases like measles and influenza, characterized by rapid spread and explosive epidemics, large amounts of virus are excreted by patients for brief periods of several days. In the case of respiratory syncytial virus infections, more than 100,000 infectious units per milliliter of nasopharyngeal washings are present for two to three weeks after onset; thus, the virus is highly contagious and outbreaks may last several months.

In the case of infectious mononucleosis, identification of asymptomatic oropharyngeal excretion and the prolonged carrier state of EBV following clinical illness explains a number of questions about its transmissibility and the difficulty in tracing case-to-case spread. In age groups in which salivary exchange is high, transmissibility is high and conversely is low in groups where this type of contact is uncommon.

Other sites of EBV production and excretion have not yet been identified, and virus has not been recovered from urine, feces, and cervical secretions [22]. However, longitudinal studies similar to those of Buddingh et al. which demonstrated intermittent appearance of herpes-simplex virus in the feces of human carriers need to be carried out [23]. Iatrogenic sources of primary infection have also been recognized; transfusion of fresh whole blood, plasma, or packed red blood cells, and transplantation of bone marrow entail risk of infection to the susceptible recipient [24,25,26].

Important information is now emerging on shedding of the virus in immunologically altered hosts. Strauch et al. studied oropharyngeal EBV excretion in 80 patients from a hospital renal disease unit [27]. In antibody-positive renal transplant recipients treated with immunosuppressive agents the rate of excretion was 52 percent. In eight older seropositive individuals receiving renal homografts none were excreting EB virus in single oropharyngeal samples before transplantation; two were shedding virus 8 and 34 days after transplantation and institution of therapy. EB virus excretion in immunosuppressed patients presumably results from reactivation and release of endogenous virus remaining from previous infections. In such patients the shift fron non-productive to productive infection occurs in the presence of circulating antibody indicating that humoral antibody does not exert a regulatory role in oropharyngeal excretion and that immunosuppressive drugs may affect cell-mediated immune mechanisms. An alternative hypothesis is that such drugs act directly on oropharyngeal cells which carry the agent and cause the virus to be reactivated.

The association of immunosuppressive drugs with reactivation of latent EB virus infections provides important biologic clues concerning mechanisms of asymptomatic excretion of EB virus in the general population, whereby a reservoir of transmissible virus is maintained. In regard to transmission of EBV infection, we know that once primary infection has occurred the individual carries EBV for life. Small amounts of virus are present in the saliva of approximately 20 percent of healthy seropositive subjects, and virus-neutralizing, VCA, and EBNA antibodies persist indefinitely. However, a much clearer understanding at a molecular level is needed of the events involved in EB virus production and release in the oropharynx and perhaps elsewhere. Such complex viral-host arabesques provide challenging areas of investigation involving virologic, immunologic, and genetic techniques which may well enlarge the spectrum of EBV-associated syndromes.

REFERENCES

1. Henle G, Henle W, Diehl V: Relation of Burkitt's tumor-associated herpes-type virus to infectious mononucleosis. Proc Natl Acad Sci USA 59:94–101, 1968
2. Niederman JC, McCollum RW, Henle G, et al: Infectious mononucleosis: Clinical manifestations in relation to EB virus antibodies. JAMA 203:205–209, 1968
3. Niederman JC, Evans AS, Subrahmanyan L, et al: Prevalence, incidence and persistence of EB virus antibody in young adults. New Eng J Med 282:361–365, 1970
4. Evans AS, Niederman JC, McCollum RW: Seroepidemiologic studies of infectious mononucleosis with EB virus. New Eng J Med 279:1121–1127, 1968
5. Biggar RJ, Henle W, Fleischer G, et al: Primary Epstein-Barr virus infections in African infants. 1. Decline of maternal antibodies and time of infection. Int J Cancer 22:239–243, 1978
6. Lang DJ, Garruto RM, Gajdusek DC: Early acquisition of cytomegalovirus and Epstein-Barr virus antibody in several isolated Melanesian populations. Amer J Epid 105:480–487, 1977
7. Henle G, Henle W: Observations on childhood infections with Epstein-Barr virus. J Inf Dis 121:303–310, 1970
8. Joncas J, Mitnyan C: Serological response of the EBV antibodies in pediatric cases of infectious mononucleosis and in their contacts. Can Med Assoc J 102:1260–1263, 1970
9. University Health Physicians and PHLS Laboratories, A joint investigation: Infectious mononucleosis and its relationship to EB virus antibody. Br Med J 4:643–646, 1971
10. Hallee TJ, Evans AS, Niederman JC, et al: Infectious mononucleosis at the United States Military Academy. Yale J Biol Med 47:182–195, 1974
11. Sawyer RN, Evans AS, Niederman JC, et al: Prospective studies of a group of Yale University freshmen. I. Occurrence of infectious mononucleosis. J Inf Dis 123:263–270, 1971
12. Wising PJ: A study of infectious mononucleosis (Pfeiffer's disease) from the etiological point of view. Acta Med Scand (suppl 133) 1:507–512, 1942
13. Hoagland RJ: The transmission of infectious mononucleosis. Amer J Med Sci 229:262–272, 1955

14. Chang RS, Golden HD: Transformation of human leucocytes by throat washings from infectious mononucleosis patients. Nature (London) 234:359-360, 1971
15. Gerber P, Goldstein LI, Lucas S, et al: Oral excretion of Epstein Barr viruses by healthy subjects and patients with infectious mononucleosis. Lancet ii:988-989, 1972
16. Miller G, Niederman JC, Andrews L: Prolonged oropharyngeal excretion of Epstein-Barr virus after infectious mononucleosis. New Eng J Med 288:229-232, 1973
17. Periera MS, Field AM, Blake JM, et al: Evidence for oral excretion of EB virus in infectious mononucleosis. Lancet i:710-711, 1972
18. Lipman M, Andrews L, Niederman JC, et al: Direct visualization of enveloped Epstein-Barr herpes virus in throat washing with leukocyte-transforming activity. J Inf Dis 132:520-523, 1975
19. Niederman JC, Miller G, Pearson HA, et al: Infectious mononucleosis. Epstein-Barr-virus shedding in saliva and the oropharynx. New Eng J Med 294:1355-1359, 1976
20. Lemon SM, Hutt LM, Shaw JE, et al: Replication of EBV in epithelial cells during infectious mononucleosis. Nature 268:268-270, 1977
21. Morgan DG, Niederman JC, Miller G, et al: Site of Epstein-Barr virus replication in the oropharynx. Lancet ii:1154-1157, 1979
22. Visintine AM, Gerber P, Nahmias AJ: Leukocyte transforming agent (Epstein-Barr virus) in newborn infants and older individuals. J Pediat 89:571-575, 1976
23. Buddingh CJ, Schrum DI, Lanier JC, et al: Studies of the natural history of herpes simplex infections. Pediat 11:595-610, 1953
24. Blacklow NR, Watson BK, Miller G, et al: Mononucleosis with heterophile antibodies and EB virus infection acquired by an elderly patient in the hospital. Am J Med 51:549-552, 1971
25. Turner AR, MacDonald RN, Cooper BA: Transmission of infectious mononucleosis by transfusion of pre-illness plasma. Ann Int Med 77:751-753, 1972
26. Sullivan JL, Wallen WC, Johnson FL: Epstein-Barr virus infection following bone marrow transplantation. Int J Cancer 22(2):132-135, 1978
27. Strauch B, Siegel N, Andrews L, et al: Oropharyngeal excretion of Epstein-Barr virus by renal transplant recipients and other patients treated with immunosuppressive drugs. Lancet i:234-237, 1974