Infectious Mononucleosis

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Preface

I am a neophyte in the study of infectious mononucleosis (IM). I did not get seriously involved until 1968, when the Henles described the first major clue to the etiologic relationship between the Epstein-Barr virus (EBV) and IM. Once involved, I became fascinated by the epidemiologic, clinical, and laboratory aspects of IM. Being a neophyte has at least one advantage—the neophyte is able to study the disease with an open mind, without preconceived notions, and with no stance that has to be defended.

I read and reread Hoagland's authoritative monograph on IM published in 1967, during the pre-EBV era. He emphasized the monotonous regularity with which a few basic signs and symptoms recurred in IM patients. Yet, other clinicians described IM as a disease with protean manifestations and with the capability of simulating diseases of many organ systems. Which notion is correct? Perhaps both are correct—the difference may be due entirely to the investigators' emphasis on either classic or more exceptional cases. Looking at Hoagland's stringent criteria for the diagnosis of IM, I am impressed by the usefulness of his criteria in an era when IM was a disease of unknown etiology, but I cannot help wondering whether his criteria may not be too restrictive in the post-EBV era. Hoagland used the term *infectious mononucleosis* rather than *glandular fever* presumably because of his belief that IM is infectious. Yet, he freely acknowledged that the epidemiologic feature of IM was inconsistent with classic infectious disease. Can we resolve this apparent contradiction in the post-EBV era?

The symposium volume on IM, edited by Carter and Penman and

published in 1969, underscored two additional facts: IM is a self-limited lymphoproliferative disease, and IM patients produce a large number of abnormal antibodies, some of which react against the host's own tissue. Can this self-limited lymphoproliferation become a progressive one under certain host and/or environmental conditions, and how does the host produce an array of antibodies totally unrelated to the EBV? Can we find satisfactory answers from current immunologic concepts?

As a physician with considerable background in public health, and with a chosen career as a medical educator who also does laboratory research, I have attempted to read as many publications as I can about IM. I must confess that I am overwhelmed by the literature. Not only is the number of publications large, but the spectrum of interest is wide. Literature search through MEDLARS yielded over 800 publications in English between July 1975 and June 1978 on the EBV and IM! These publications appeared in a range of journals from the very basic *(Journal of Molecular Biology)* to the very clinical *(Age and Ageing)*. Even psychiatric journals contain articles on IM.

This new monograph on IM can serve two useful purposes. First, it provides an up-to-date summary of the latest (to 1979) information on IM, and second, it catalogs references that give in-depth information on specific aspects of IM.

Reviewing the vast amounts of information contained in the references enabled me to discern relationships. For example, certain biological attributes of the EBV appear to cause certain pathological findings which, in turn, appear to be responsible for certain clinical manifestations of IM. Through our knowledge of the biological attributes of the EBV, we can almost describe steps in the pathogenesis of IM and predict its clinical manifestations. With knowledge of the EBV and its interaction with the host, we can explain why IM is what it is. Medical educators are acutely aware of the difficulty in motivating some medical students to learn basic medical sciences because of their "irrelevance" to clinical medicine. Study of the disease IM bridges the "relevance" gap because in it the relation of the basic to the clinical sciences is readily perceived.

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Contents

Chapter 1.

The Epstein-Barr Virus

1.1 Discovery

Based on his extensive epidemiologic observations, Burkitt proposed the provocative hypothesis that a lymphoma syndrome among children in tropical Africa might be caused by an arthropod-borne virus (1963). This hypothesis generated a colossal volume of research on this tumor, now known as *Burkitt's lymphoma*. One type of research was the examination of Burkitt's lymphoma tumor tissue for viruses. Several viruses were found (Simons and Ross 1965; Bell et al. 1966; Woodall et al. 1965); one of them is a herpesviruslike particle, now known as the *Epstein-Barr virus* (EBV).

Pulvertaft (1964) and Epstein and Barr (1964) successfully propagated lymphoid cells from biopsies of Burkitt's lymphoma. Examining thin sections of these lymphoid cells with the electron microscope, Epstein, Achong, and Barr (1964) and Epstein and associates (1965) found herpesviruslike particles which were biologically and antigenically distinct from any of the human herpesviruses known at that time. Soon after, continually multiplying lymphoid cells were successfully isolated from other Burkitt's lymphomas (Steward et al. 1965; Rabson et al. 1965), from the blood and lymph nodes of patients with leukemia or solid cancers (Iwakata and Grace 1964; Zeve, Lucas, and Manaker 1966; Jensen et al. 1967), from the blood and bone marrow of infectious mononucleosis (IM) patients (Gerber and Birch 1967*b*; Pope 1967; Diehl et al. 1968; Moses et al. 1968; Benyesh-Melnick et al. 1968) and from the blood of healthy persons (Moore, Gerner, and Franklin 1967; Gerber and Monroe 1968). Herpesviruslike particles were also found in

the cell lines in some of the continually multiplying lymphoid cells. To determine the antigenic relatedness of these herpesviruslike particles, Epstein and Achong immunized rabbits with virus particles from a line of Burkitt's lymphoma cells and tested the antiserum on viruses derived from five other sources (1968*a*;*b*). The viruses were all related antigenically. It was proposed that this group of antigenically related herpesviruslike particles, found in human lymphoid cells derived from diverse sources, be named the Epstein-Barr virus.

Taking advantage of the facts that the EBV can transform human lymphocytes, that the EBV is implicated as the etiologic agent of IM, and that IM is sometimes referred to as the "kissing disease," Chang and Golden tested the oropharyngeal secretions of IM patients for leukocyte-transforming activity. They reported in 1971 that the throat washings of IM patients contained a filterable agent capable of transforming human peripheral leukocytes into rapidly and persistently multiplying lymphoid cells. Further studies (Gerber et al. 1972; Chang et al. 1973; Golden et al. 1973; Chang, Lewis, and Abildgaard 1973; Strauch et al. 1974; Sumaya 1975) showed that many persons not suffering from IM were also excreters of this leukocyte-transforming agent. The key question is whether the transforming agent is the EBV. Because it is difficult to propagate the transforming agent in human leukocyte culture or any tissue-culture system, sufficient quantities of it cannot be obtained for direct-identification studies (Chang, Lewis, and Abildgaard 1973).

Given the difficulty of propagating the leukocyte-transforming agent, its identification has to be approached by indirect means through the study of leukocytes transformed by it. Pereira and associates found an occasional herpesviruslike particle in a transformed cell (1972). Gerber and associates (1972) and Pagano (1975) found in the transformed cell a deoxyribonucleic acid (DNA) sequence homologous to the DNA of a strain of EBV. Strauch and associates found EBV antigens in the transformed cell (1974). Miller, Niederman, and Andrew neutralized the leukocyte-transforming activity of the transforming agent with two human sera that contained EBV antibody but not with two human sera that did not contain EBV antibody (1973). Lipman and associates described seeing a herpesvirus in a throat washing with transforming activity (1975). Considering all the indirect evidence, we can say that the transforming agent is probably the EBV.

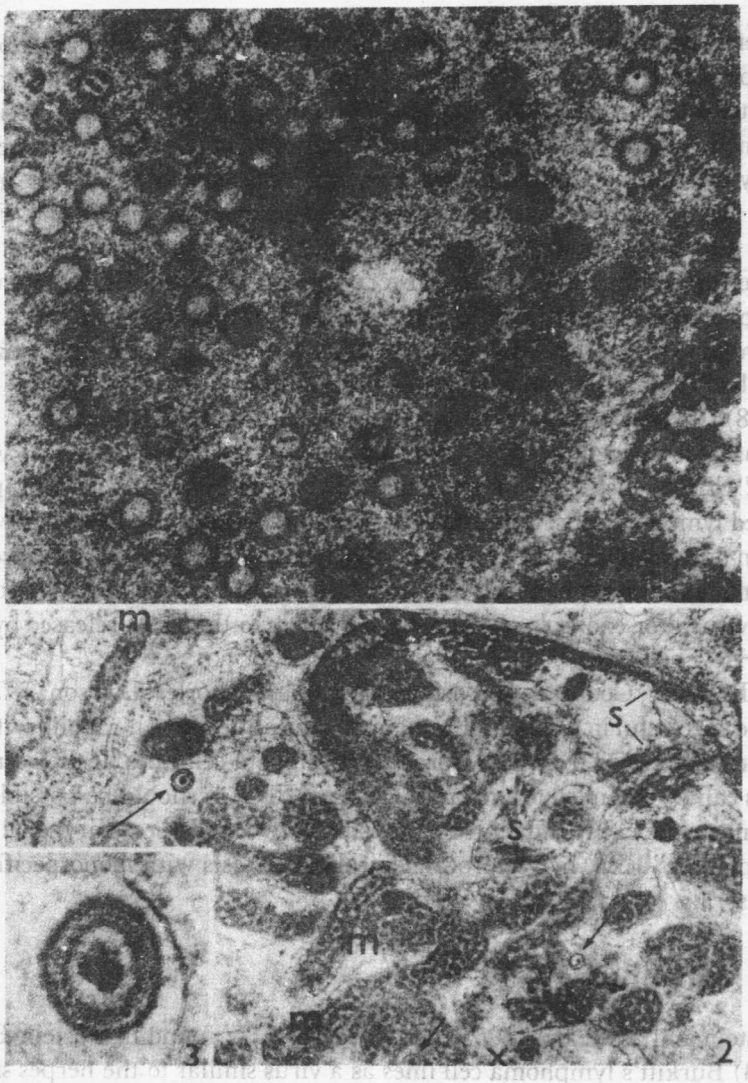
What must be done to prove conclusively that the oropharyngeal transforming agent is the EBV? We must show that the transforming

potency of throat washings varies proportionately with the number of herpesviruslike particles present. We must also show the immunologic relatedness of the transforming agent and the prototype EBV by reciprocal neutralization tests using monospecific antisera obtained from specifically immunized animals rather than from selected humans. There are two reasons why these simple experiments have not yet been done. First, it is difficult to obtain a sufficient quantity of the transforming agent for experimentation because the transforming agent propagates poorly in vitro. Second, it is difficult to get monospecific sera because rabbits immunized with the EBV apparently fail to form a specific neutralizing antibody (Hampar and associates 1970; Vestergaard and associates 1978). Until conclusive evidence is obtained, it is proposed that the transforming agent in throat washings be referred to as the *oropharyngeal EBV* to distinguish it from the EBV found in continuously propagated lymphoid cells. Since there is no assurance that the EBV derived from one lymphoid cell line is necessarily identical to the EBV derived from another lymphoid cell line, it is also useful to indicate the source of the virus. For example, *P3HR-1 EBV* refers to the EBV released by the P3HR-1 cell line.

Recently, Coope and associates (1979) and Thorley-Lawson (1979) were successful in raising EBV-neutralizing antibodies in rabbits with materials derived from EBV-producer lymphoid cell lines. If their findings can be extended to the oropharyngeal EBV, it should be possible to delineate the exact antigenic relationship between the oropharyngeal and "prototype" EBV by reciprocal neutralization tests with monospecific antisera.

1.2 Morphology

Epstein and associates (1965) described the EBV found in Epstein-Barr (EB) Burkitt's lymphoma cell lines as a virus similar to the herpes simplex virus in appearance except for its smaller size (figs. 1.1–1.3). Cross sections of the virion reveal a central dense core surrounded by two membranes. The diameters of the central core (nucleoid), inner membrane (nucleocapsid) and outer membrane (mature virion) are 45–50, about 75, and 110–115 nm, respectively. Nucleocapsids are found primarily in the nucleus or cytoplasm of degenerating rather than healthy cells. Mature virions are always surrounded by a fine membrane within the cytoplasm. Toplin and Schidlovsky studied partially purified virus from



Figs. 1.1–1.3. Electron micrographs of thin-sectioned Epstein-Barr virus-transformed cells derived from Burkitt's lymphoma. In figure 1.1 numerous capsids and nucleocapsids are visible in the nucleus. In figure 1.2 a mature virion (long arrow) is seen within a membrane-bounded cytoplasmic space (m = mitochondria; s = spindle tubule; x = a space into which a virus particle appears to be maturing by budding). Figure 1.3 shows a detail of a mature virion. Magnifications: figure 1.1 76,500x; figure 1.2 42,000x; figure 1.3 213,500x (Epstein et al. 1965).

the EB-3 cell, confirmed Epstein and associates' finding, and further showed that the mature virion was enveloped and contained a nucleocapsid with icosahedral symmetry (1966). The number of capsomers per nucleocapsid is estimated at 162. The EBV released by the B95-8 marmoset cell line (Miller et al. 1974) and the oropharyngeal EBV (Lipman et al. 1975) are morphologically similar to the EBV found in EB-3 cells (Epstein et al. 1965). Miller and associates subsequently found the median diameter of the EBV nucleocapsid to be 110 nm rather than 75 nm (1974).

1.3 Physicochemical characteristics

EBV released by the EB-3 cell has a density of 1.19–1.21 g/cm^3 (Toplin and Schidlovsky 1966). The genome of virions released by the P3HR-1 lymphoma cell (Hinuma et al. 1967*a*) and the B95-8 marmoset cell (Miller and Lipman 1973) is a linear molecule of double-stranded DNA with a density of 1.718–1.720 g/cm^3, a molecular weight of about 10^8 daltons, and a guanine-cytosine content of 57–59% (Schulte-Holthausen and zur Hausen 1970; Pritchett, Hayward, and Kieff 1975; Sugden, Summers, and Klein 1976; Adams et al. 1977). Results of a nucleic-acid hybridization study showed that the P3HR-1 virus had over 97% of the DNA sequences of the B95-8 virus, but the B95-8 virus lacked about 15% of the sequences of the P3HR-1 virus (Pritchett, Hayward, and Kieff 1975). Another study showed that the P3HR-1 and B95-8 EBV shared about 90% of their nucleotide sequences (Sugden, Summers, and Klein 1976). The DNA from these two EBVs are clearly not identical.

Preliminary analysis of proteins from the P3HR-1 and B95-8 virions revealed at least 33 molecular species of polypeptides with molecular weights from 28,000–290,000 daltons (Dolyniuk, Pritchett, and Kieff 1976). Being an enveloped virion, the infectivity of the EBV is destroyed by ether (Pope, Horne, and Scott 1969).

1.4 Biological characteristics

The four important biological characteristics of the EBV are its ability to: (1) transform human lymphoid cells, (2) induce lymphoma in marmosets, (3) cause IM in humans, and (4) persist in an infected host. These characteristics are discussed in chapters 2, 3, 4, and 10.