CHAPTER 9

Epstein-Barr Virus

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1. Introduction

Epstein-Barr virus (EBV), a member of the herpes group of viruses, is the cause of heterophil-positive infectious mononucleosis, of some heterophil-negative cases, and of occasional cases of tonsillitis and pharyngitis in childhood. Rarely, it may produce involvement of the central nervous system. This virus is strongly implicated as having a causal relationship to African Burkitt lymphoma and to nasopharyngeal cancer. High antibody titers are also present in 40–50% of cases of Hodgkin's disease, sarcoidosis, and systemic lupus erythematosus.

This chapter will deal with the epidemiology of EB virus infections and the epidemiology of infectious mononucleosis. It will also mention the relationship of high antibody titers to certain chronic and malignant diseases, but the major discussion of Burkitt lymphoma and of nasopharyngeal cancer will be found in later chapters of this book.

2. History

In 1889, Emil Pfeiffer of Wiesbaden, Germany, described a condition called *Drüsenfieber* (glandular fever), characterized by fever, adenopathy, mild sore throat, and in severe cases enlargement of the liver and spleen.[126] As this description antedated by some 30–50 yr the recognition of the hematological changes and the discovery of heterophil antibody, it is uncertain whether this can be accepted as true infectious mononucleosis. However, his description of this type of febrile syndrome in older children and young adults seems best to fit this diagnosis. There is little doubt about the classical description of the disease made by Sprunt and Evans, from Johns Hopkins, in 1920.[139] They described the disorder in young adults as we now know it, named the disease "infectious mononucleosis," and reported in detail the hematological changes. This description was followed rapidly by similar reports from other workers.[12,22,96,108] A definitive presentation of hematological changes was made by Downey and McKinlay in 1923.[28] The next major development was the discovery in 1932 of the heterophil antibody by Drs. John R. Paul and William W. Bunnell of Yale University.[121] Their report was based on an accidental observation while studying the occurrence of heterophil antibodies in rheumatic fever. This search had been initiated because of the clinical similarity of rheumatic fever and serum sickness and because of the work of Davidsohn[24] describing the presence of heterophil antibodies in serum sickness. Among the control subjects for rheumatic fever patients was one who had infectious mononucleosis and was found to have a much higher heterophil antibody titer than present in any other condition. Paul and Bunnell then continued these observations in three additional cases of infectious mononucleosis and utilized 275

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controls for comparison. Their paper also describes what they believed to be a false-positive heterophil antibody occurring in a patient with aplastic leukemia. A review of the details of this case[43] reveals that the heterophil antibody occurred about 20 days after the administration of several units of blood and therefore this patient may represent the first case of transfusion infectious mononucleosis. Soon after the discovery of the presence of heterophil antibodies, Davidsohn and Walker[23] reported on the use of guinea pig kidney and of beef cells to absorb serum prior to heterophil testing in order to increase the specificity of the test. Both of these procedures have withstood the test of time well and still constitute one of the major criteria of diagnosis. Regular alterations in various liver function tests during acute infectious mononucleosis were recognized in several laboratories in the late 1940s and 1950s,[13,35,86] even though only 5% of patients had clinical jaundice. This was followed by the discovery of alterations in serum glutamic oxalacetic transaminase (SGOT) and other hepatic enzymes during the course of disease.[6,131,158]

Search for the etiological agent of infectious mononucleosis began in the 1920s, but met little success until 1942 when Wising[156] reported the successful transmission of classical infectious mononucleosis to a female medical student volunteer who received 250 ml of blood from a patient ill with the acute disease. This successful experiment was not reproducible by Wising in several other attempts, nor by Bang,[3] who carried out a similar set of volunteer experiments. In 1947 and again in 1950, additional efforts of this sort were carried out at Yale University using whole blood, serum, or throat washings. The results provided suggestive but inconclusive evidence of transmission.[34,36] A third effort with questionable success was reported from Yale University in 1965.[112] Subsequent EBV antibody tests on the sera from these last experiments in 1968 revealed that all volunteers had actually been immune to infection prior to the experiment as indicated by the presence of antibody.[111]

Repeated attempts in the 1950s to isolate an infectious agent from the throat or blood of patients with infectious mononucleosis using several tissue culture systems, long-term cultures of lymphocytes on a feeder layer, and fluorescent antibody techniques to identify an agent were unsuccessful.[37] Epidemiologically, the key events during this time were the observations of Hoagland, who suggested that the disease might be transmitted by kissing[79] and that the incubation period was of the order of 30–49 days.[81]

Early in 1968, evidence first appeared that EB virus was the cause of infectious mononucleosis.[72,114] This virus, isolated by Epstein, Barr, and Achong from a culture of African Burkitt tumor tissue, was found to be a new member of the herpes group of viruses.[33] While working with this agent, a technician in the Henles' laboratory in Philadelphia developed infectious mononucleosis. Her serum, which lacked antibody several months prior to disease, developed EBV antibody during illness, and her lymphocytes, which had failed to be cultivated successfully hitherto, now grew well in tissue culture and were shown to contain EB viral antigen.[72] This serendipitous observation was rapidly confirmed and extended at that time by the Henles in conjunction with Niederman and McCollum of Yale[114] and later in several other prospective studies carried out by the Yale team[50,113,132] and in one English study.[148] Subsequent investigations established the presence and persistence of EB virus in the throat during and after acute infectious mononucleosis,[19,59,103] the occurrence of EBV-specific IgM,[4,66,114a,133] and the reproduction of some features of mononucleosis by inoculation of EB virus into monkeys.[135,154] Fuller details of the history of infectious mononucleosis have been published elsewhere.[16,43]

3. Methodology

3.1. Mortality Data

Infectious mononucleosis is rarely a fatal disease; only about 50 fatalities have been reported.[39] Examination of autopsy records or of international indexes of causes of death would therefore give little indication of the occurrence of the disease even though its pathological features are quite characteristic.

3.2. Morbidity Data

Infectious mononucleosis is not a reportable disease in most states and in most countries. Exceptions are the state of Connecticut, where it has been reportable since 1948,[21] and the U.S. armed forces, which collect hospitalization data on all diseases.[41] Unless strong emphasis is placed on the need for fulfilling clinical, hematological, and serological criteria for diagnosis before reporting, the reliability of morbidity data from these sources must be seriously questioned. This requirement is emphasized by the fact that even for the 15–25 age group—where the disease has its highest incidence, its most characteristic clinical features, and the highest frequency of elevated heterophil antibody tests—only one-third of the serum samples sent to a state laboratory for diagnosis of suspected cases were heterophil antibody positive.[38]

In order to collect morbidity data, special surveys of selected populations for infectious mononucleosis have been carried out in college infirmaries,[50,113,132] community medical care groups,[69] general practitioners' offices,[83] and by physicians and laboratories serving defined communities.[38,68] The Center for Disease Control also periodically publishes a Surveillance Report on infectious mononucleosis based on data derived from 19 colleges.[18]

The problems of data derived from such surveys are related to the extent to which the numerator or case report reflects the proper diagnosis and whether adequate surveillance has been carried out with respect to the denominator—the population at risk.

3.3. Serological Surveys

Up to 1968, when the causal association of EB virus to infectious mononucleosis was discovered, the heterophil antibody constituted the only serological approach to diagnosis and survey work. Because this is an IgM-type antibody and is transient in nature, it can be used as a serological tool only for incidence data—i.e., during the acute illness, and as an essential diagnostic feature.[114] The specificity of a properly performed quantitative heterophil antibody test is high provided that the serum has been preabsorbed with guinea pig kidney in the sheep and horse red cell tests, or that the beef hemolysin test has been used. In a test performed in this way, an elevated titer quite accurately reflects the occurrence of infectious mononucleosis even in the absence of clinical and hematological data and has been utilized as an indicator of infectious mononucleosis in sera sent to hospitals and state laboratories.[38] The major limitation of this approach is the extent to which physicians have sent sera from suspected cases to the diagnostic laboratory for analysis. The increasing use of simple laboratory kits for identifying heterophil antibody elevations in the physician's office probably results in much less utilization of state and hospital laboratories so that morbidity data from these sources may greatly underestimate the occurrence of the disease. Thus, utilization of the heterophil antibody as an epidemiological tool to identify the acute illness has high reliability and specificity but low sensitivity. Heterophil-positive cases diagnosed in the Connecticut State Public Health Laboratory alone represented 74.5% of all the reported cases in the state in 1972.

Since the discovery of EB virus as the cause of infectious mononucleosis in 1968, many serological surveys in different countries have been made for the presence of antibody to this virus in sera collected from healthy persons, usually employing the indirect immunofluorescence test[70] for viral capsid antigen. This antibody persists for many years, perhaps for life.[113,114] These studies yield prevalence data on prior EBV *infection* but give no direct indication of the occurrence of clinical infectious mononucleosis.

The most accurate information on the *incidence* of both EBV infections and clinical infectious mononucleosis has come from prospective seroepidemiological studies of defined populations with close clinical surveillance for the occurrence of suspected cases of infectious mononucleosis and other illnesses. Sera taken at the start of the observation period are tested to define the number of susceptibles, i.e., those lacking EBV antibody; samples showing seroconversion at the end of the observation period will identify the total EBV infection rate; those collected during interim illnesses and tested for EBV and heterophil antibodies will delineate the number and spectrum of

clinical illnesses, such as infectious mononucleosis, associated with EBV infection.

3.4. Laboratory Methods

3.4.1. EBV Isolation. The virus cannot be grown in the usual tissue cultures employed for other herpesviruses. The currently available isolation technique is tedious, difficult, and usually confined to research laboratories. It is based on the ability of EB virus to transform uninfected human leukocytes into continuous cell lines and the identification of this effect as due to EBV.[19,103] Leukocytes derived from the cord of newborn infants or from persons lacking EBV antibody are employed to ensure absence of EBV antigen in the lymphocytes. Throat washings or other materials to be tested are usually filtered to remove debris and bacteria, then added to the leukocytes, and placed on a placental fibroblast feeder layer. If EBV is present, evidence of transformation is indicated by an abrupt increase in the total number of cells, the production of acid, growth of cells in clumps, and the development of the capacity to be subcultured indefinitely. Usually, transformation occurs 30–90 days after addition of the throat washing.

The presence of EBV nuclear antigen can be demonstrated in acetone-fixed smears of transformed cord cells using an indirect complement fixation fluorescence technique,[129] but viral capsid antigen (VCA) cannot be demonstrated by ordinary immunofluorescence methods, presumably because the virus does not mature sufficiently in such cells. EBV-transformed leukocytes may also be grown further in culture to prepare a complement-fixing antigen as a means of identification; however, this is a laborious method. More sensitive methods of antigen detection, chemical means of enhancing the rapidity of viral multiplication to shorten the long observation time, or discovery of a more sensitive cell line will be needed before viral isolation will be practical in the diagnostic laboratory. Recently, Robinson and Miller[129a] have demonstrated that DNA stimulation occurs early in EBV infected cord cells and can be detected by increased uptake of radioactive-labeled thymidine.

3.4.2. EBV Antibody. A wide variety of techniques to measure EBV antibody have been developed. Five antibody methods based on immunofluorescence have been used. First, for epidemiological purposes the indirect immunofluorescence test of the Henles[70] for viral capsid antigen has been widely employed in serological surveys as a reliable indicator of susceptibility and immunity to infectious mononucleosis. However, it has not proved very useful as a diagnostic test for infectious mononucleosis because antibody is usually present by the time the patient seeks medical care and rises in titer are detectable in only 15–20% of cases. Second, antibody to "early antigen" is also identified by immunofluorescence techniques[74,76] and its presence is indicative of recent or active infection. Unfortunately from a diagnostic standpoint it is demonstrable only in about 75% of patients with infectious mononucleosis[77]; it also occurs in the sera from patients with Burkitt lymphoma and nasopharyngeal cancer. Third, antibodies to Epstein-Barr virus associated nuclear antigen (EBNA) are detectable by an immunofluorescence technique based on fixation of complement; these usually arise only 1 or more months after onset of infectious mononucleosis and after primary infections and probably persist for life.[73] Their late appearance impairs their usefulness in routine diagnosis. Fourth, there is an indirect immunofluorescence test for EBV-specific IgM antibody, and this is the most useful procedure for the diagnosis of heterophil-negative infectious mononucleosis[29,114a,133]; however, in its present form this test is technically difficult to perform and interpret, and it is thus not currently available in diagnostic laboratories. Finally, a membrane fluorescence test has been developed by Klein *et al.*[88,89,90] Other antibody tests include complement fixation using either the virus[1] or soluble antigens,[57] neutralization tests based on contact inhibition,[78,105] and immunodiffusion tests.[116]

3.4.3. Heterophil Antibody Tests. Three general methods are employed to test for heterophil antibody: (1) the classic Paul-Bunnell test[121] using sheep or horse red cells after absorption of the serum with guinea pig kidney as developed by Davidsohn and Walker[23]; (2) the beef cell hemolysin test of Bailey and Raffel[2] adapted for diagnostic use by Mason,[98] which does not require absorption of sera with guinea pig kidney because beef cell hemolysins are absent or at very low titers in normal sera; and (3) the enzyme test of

Wöllner,[157] in which red cell receptors for heterophil antibody are specifically removed by treatment with papain or a similar enzyme. Recent evidence suggests that heterophil antibody titers may reach diagnostic levels even after mild or asymptomatic EBV infections provided that serial specimens are tested over a month or so by the horse cell differential test.[52] The test may be useful in childhood EBV infections, which are often mild. Most state and large diagnostic laboratories employ either the Davidsohn differential absorption test or the beef cell hemolysin test; the former, using horse cells, is more sensitive, and heterophil antibody to this antigen often persists at diagnostic levels for as long as a year or so.[52,93] The beef hemolysin antibody is more specific but disappears in 3 months or less; it is perhaps the most reliable test to diagnose infectious mononucleosis during the acute illness.

A number of commercial testing kits for the diagnosis of infectious mononucleosis in the physician's office are now available. Most are slide agglutination tests, usually performed at a single dilution and commonly based on the agglutination of formalinized horse cells. Some of these employ guinea pig kidney to remove nonspecific agglutinins from the serum prior to testing. Other tests employ papain-treated red cells in the agglutination test. Such tests are useful if carried out by trained personnel using fresh material; they should be used for corroboration of clinical and hematological findings, and if doubt exists should be confirmed by a standard quantitative test.

4. Biological Characteristics of the Agent

4.1. The Virus

Epstein-Barr virus is a distinctive member of the herpes group of viruses. On electron microscopy, EBV appears similar to other herpes-group viruses.[33] Currently, two laboratory strains of EBV have been identified,[106] but the sophisticated techniques needed to differentiate strain differences as small as exist between herpes types I and II are not yet available. The virus has been cultivated only in suspension cultures of primate lymphocytes, and most cultures yield only small amounts of extracellular virus. These limitations have made characterization of the physical and chemical properties of EBV very difficult. All lymphocytes in continuous cultures established from infectious mononucleosis blood or Burkitt lymphoma biopsies contain the EBV genome as demonstrated by DNA hybridization or EBV nucleic acid (EBNA) tests, but only 1–3% have demonstrable viral capsid antigen[115,129,160]; cell clones grown from such cultures show a similar low percent of complete virus.[99,159] One line of cells from Burkitt lymphoma, the P3J line of Pulvertaft,[128] and its cloned derivative, the HR1K, produce more extracellular virus than other lines. Another line derived from EBV-infected marmoset cells by Miller and Lipman[102] releases about 1000 times more transforming virus but about the same number of viral particles as HR1K. It should be useful in viral characterization.

Some of the biological properties of EBV are important epidemiologically. The capacity for persistence of a lytic infection in the throat provides a source of potential transmission; the low yield of extracellular virus may bear on the need for intimate, oral contact, perhaps with transfer of infected cells, for transmission in young adults. The reasons for the higher efficiency of transmission of EBV infection in young children than in adults are unknown but might include the production of more infectious virus in the pharynx of children, more intense exposure, or spread by a fecal–oral route in settings with poor hygiene.

The capacity for persistence and latency of EBV in a *nonproductive* form sets the stage for later reactivation under conditions of immunosuppression (malaria, therapeutic immunosuppression in renal transplants, etc.). African Burkitt lymphoma and nasopharyngeal cancer may be expressions of this reactivation. The long-term persistence of EBV in lymphocytes is of importance epidemiologically in the transmission of infection during blood transfusions to susceptible recipients. Of great importance is the capacity of EBV to transform uninfected primate lymphocytes, inducing in them the potential for unlimited proliferation, a property termed "immortalization" by Miller,[100] and the lymphocytes that result are termed "I-lymphocytes." The EBV-transformed and infected cells appear to be B-type lymphocytes.[118,119] Viral induction of new antigens (or unmasking of preexisting ones) such as the membrane antigen of

Table 1. Summary of 11 Prospective Studies of EB Virus Infection in Children and Young Adults[a]

EBV antibody status at start	Number	Percent	Subsequent rate per 100/yr	
			EBV infection	Clinical infectious mononucleosis[b]
With antibody	3733	70.7	0	0
Without antibody	1547	29.3	16.4	7.1
Total	5280	100	4.6	2.0

[a] From ten studies carried out by Yale investigators and one by an English team.[148]
[b] Clinical infectious mononucleosis was recognized in 47% of those infected with EBV.

Klein *et al.*[88] may have immunological consequences in the development of new antibodies, in a graft-vs.-host response, or in mixed lymphocyte type reactions with uninvolved lymphocytes.

A better understanding of the dynamics and effects of EBV activity at molecular and cellular levels ("molecular epidemiology") and of the responses of the host to them under varied conditions of age, concomitant infection, immune status, and genetic constitution will be needed before the full spectrum of clinical response is known.

4.2. Proof of Causation of Infectious Mononucleosis

The causation of heterophil-positive infectious mononucleosis by EBV has been established beyond any reasonable doubt. The proof is based on seroepidemiological and virological evidence and also on partial success in the experimental transmission of infection to monkeys and man.

Seroepidemiological investigations have repeatedly shown that *antibody* to EBV of the IgG type has been consistently absent in sera taken prior to the onset of infectious mononucleosis, regularly appears during illness, and persists for years thereafter.[50,65,114,132,148] The presence of this antibody indicates immunity to clinical infectious mononucleosis, and its absence indicates susceptibility to the disease. Table 1 summarizes 11 prospective studies involving over 5000 children and young adults in support of this relationship. No other virus has been found that induces a similar antibody and no other viral antibody has been demonstrated during heterophil-positive infectious mononucleosis. The occurrence of some heterophil-negative cases of infectious mononucleosis due to EBV has also been noted in prospective studies.[50,65] Other mono-like syndromes are due to cytomegalovirus and other agents.

EBV-specific antibody of the IgM class has been demonstrated during acute infectious mononucleosis and found to disappear during convalescence, thus indicating that this is a primary response to EBV infection.[4,29,66,133] Both the IgG and IgM EBV-specific antibodies of infectious mononucleosis are distinct from the heterophil antibody.

The virological evidence consists of the appearance of EBV in the oropharynx and in the circulating lymphocytes of patients with acute infectious mononucleosis. The agent has been regularly demonstrated in the pharynx of over 80% of patients during the acute illness.[19,59,103,125] It persists for many months, and in several cases has persisted for as long as a year or so. A chronic carrier state may exist. EBV has been regularly demonstrated in lymphocyte cultures from patients with acute infectious mononucleosis, where it may persist in a latent form for years and may be a source of transfusion mononucleosis.[58,75] EBV has not yet been demonstrated in *fresh* lymphocytes from patients with infectious mononucleosis: whether this is due to small amounts of virus, insensitive methods, existence in altered form, the destruction of EBV-infected B cells by T lymphocytes, or other

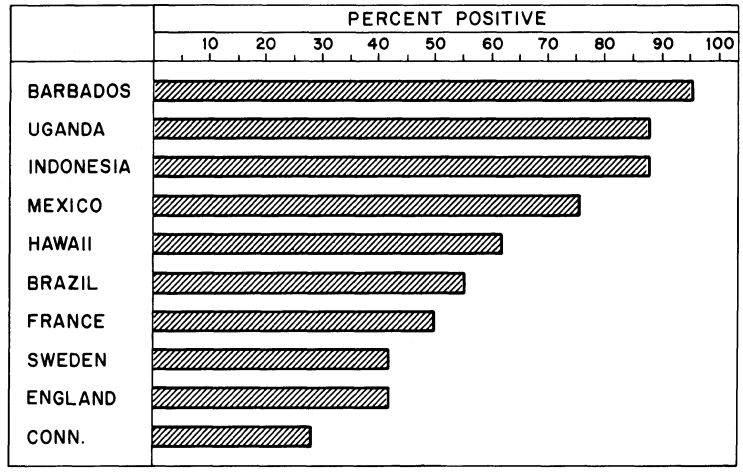

Fig. 1. EBV antibody prevalence at age 4–6 yr in different populations. Adapted from A. S. Evans, New discoveries in infectious mononucleosis, *Mod. Med.* **1:** 18–24 (1974).

reasons is currently unknown.* The appearance and persistence of EBV in the oropharynx following mild or asymptomatic infections provides a large pool of healthy carriers capable of transmitting infection through appropriate exposure.

Efforts to transmit infectious mononucleosis to volunteers using blood, throat washings, or stools from acutely ill patients were made prior to the discovery of EBV in 1968; the results were largely inconclusive or unsuccessful, probably because of the presence of prior immunity in those inoculated. However, there are a few exceptions. Wising[156] successfully transmitted the full-blown disease to a female volunteer by transfusion. Evans[37] and Taylor[145] reported suggestive evidence of successful transmission by inoculation of pooled sera from patients with acute infectious mononucleosis into patients with acute leukemia as a therapeutic effort to induce a remission; the young age of this group probably meant that some were susceptible because they had not been previously exposed. About 50 other experiments in humans were equivocal or unsuccessful.[3,34,36,112] Similarly, earlier efforts to induce infectious mononucleosis in monkeys were not rewarding.[49,137]

* EBV nuclear antigen has recently been found in B lymphocytes separated from the blood of 4 acute cases [Klein, G., Svedmyr, E., Jondal, M., and Persson, P. O., EBV-determined nuclear antigen (EBNA)-positive cells in the peripheral blood of infectious mononucleosis patients, *Int. J. Cancer*, **17**:21–26 (1976)].

Recent studies with this virus in humans have been very limited because of concern for the oncogenicity of EBV. Grace *et al.* [61] repeatedly inoculated partially purified EBV into a terminal cancer patient who lacked prior antibody; both EBV and heterophil antibodies developed. Inoculation of EBV-infected lymphocytes into gibbons has resulted in an exudative tonsillitis and the appearance of EBV antibody.[154] Shope and Miller[135] have induced transient EBV and heterophil antibody in squirrel monkeys inoculated with virus-transformed leukocytes. The current evidence of successful transmission of infectious mononucleosis to monkeys must be regarded as incomplete at this time.

In summary, the results of seroepidemiological, virological, and transmission studies in man and monkeys indicate that EBV is the cause of heterophil-positive infectious mononucleosis.

5. Descriptive Epidemiology

5.1. Prevalence and Incidence

The *prevalence of antibody* to EBV has been determined in many countries and in many age groups.[42] Fig. 1 indicates the percentage of children in several areas of the world with EBV antibody. In developing and tropical areas, most children have been infected by age 6 yr. Because

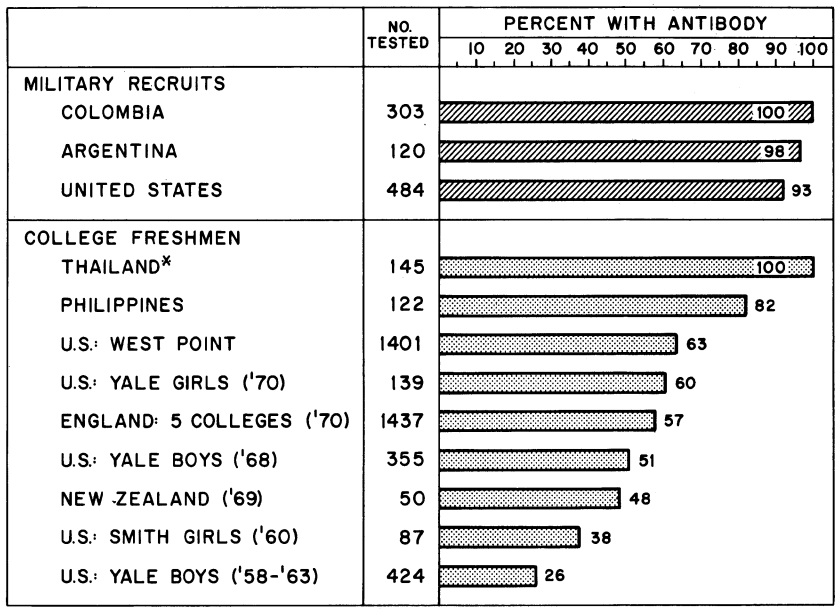

Fig. 2. EBV antibody prevalence in young adults in different populations. × Student nurses. Adapted from A. S. Evans, New discoveries in infectious mononucleosis, *Mod. Med.* **1:** 18–24 (1974).

infections with EBV are usually mild and asymptomatic in young children, it is not likely that infectious mononucleosis would be recognized as a clinical entity in such countries. The few characteristic and heterophil-positive cases that do occur may be so diluted out as to be unnoticed. The prevalence of EBV antibody in young adults living in different parts of the world is depicted in Fig. 2. A similar socioeconomic pattern exists. It is only when a significant percentage of the population reaches ages 15–25 before exposure to and infection with EBV that infectious mononucleosis emerges as an important clinical entity. This delay in exposure is largely limited to nations with high economic and hygienic levels and to middle and upper socioeconomic classes in any country. The most susceptible college group tested thus far were entering freshman students at Yale University in the period 1958–1963, when nearly 75% were at risk to infectious mononucleosis because they lacked antibody; coincident with programs that broadened admission to include students with widely differing socioeconomic backgrounds, among them many minority groups, the susceptibles decreased to 40–50%. In contrast, under 20% of undergraduate students at the University of Philippines lacked EBV antibody and all of 145 freshman student nurses in Thailand had antibody.

The *incidence of clinical infectious mononucleosis* is about 48 per 100,000 in the state of Connecticut, where the disease is reportable,[21] 45 per 100,000 according to a recent community survey in Atlanta, Georgia, of heterophil-positive cases,[68] and 200 per 100,000 in Olmstead County, Minnesota, where patients are largely handled by the Mayo Clinic.[69] Use of the rate of 48 per 100,000 in Connecticut in 1972 as generally representative would make infectious mononucleosis one of the commonest infectious diseases in the United States based on diseases summarized in the 1972 Center for Disease Control Annual Report and listed in Table 2. Data from the CDC Surveillance Report of 19 U.S. colleges place the incidence of infectious mononucleosis in this age group during 1971–1972 at 840 per 100,000[18] for college students, with a range of 485–1256.

Hospitalization rates for infectious mononucleosis in the armed forces range from 148 to 250 per 100,000.[41] In the navy and marine corps, where comparative data are available, it ranks as the fifth commonest infectious disease and the fourth commonest cause of days lost.

The most accurate measure of EBV infection and

Table 2. Estimated Number of Cases of Infectious Mononucleosis Compared to Notifiable Infectious Diseases in 1972[a]

Diseases	Number of reported cases
Gonorrhea	767,215
Chickenpox	164,114
Infectious mononucleosis[b]	97,550
Syphilis	91,149
Mumps	74,215
Infectious hepatitis	54,074

[a] From Annual Supplement, Reported incidence of notifiable diseases in the United States, 1972, CDC Morbidity and Mortality, Vol. 21 (1973).
[b] Based on rate of 48 per 100,000 in Connecticut (1972), where infectious mononucleosis is reportable, and U.S. population of 203.2 million.

disease has been obtained in prospective serological and clinical studies, where the number of susceptibles, the infection (seroconversion) rate, and the clinical attack rate can be critically defined. Comparative data are available from three prospective investigations of freshman students: at Yale University,[132] at five English colleges and universities,[148] and at the U.S. Military Academy at West Point, New York.[65] As depicted in Table 3, the incidence rate of EBV *infection* was strikingly similar in all three settings: 12–13% of *susceptible* students were infected with EBV during the freshman year, and of those with known EBV infection 27.7–74.0% developed clinical infectious mononucleosis. At the U.S. Military Academy, where a prospective investigation was carried on in a single cohort of freshmen over 4 yr, the EBV infection rate in susceptible cadets was 12.4% in the first year, 24.4% in the second year, 15.1% in the third year, and 30.8% in the fourth college year.[65] Over the 4-yr period, 45.9% of susceptible cadets were infected with EBV and 26.4% of these were known to have clinical infectious mononucleosis; others may have been ill but did not report to the clinic for treatment.

5.2. Epidemic Behavior

True epidemics of infectious mononucleosis that fulfill appropriate diagnostic criteria have not occurred in modern times.[38,82] Earlier, many purported epidemics were described, of which the most impressive are those described by West in the United States in 1896,[155] by Moir in the Falkland Islands in 1930,[107] and by Carlson *et al.* in Wisconsin in 1926.[15] More recent and suggestive outbreaks have been described from an Emergency Medical Hospital[64] and from Oxford, England, reported by Hobson *et al.*[83]

The high incidence in military camps during World War II probably reflects the rapid turnover of large numbers of men.[46,149,153] Some reported hospital "outbreaks" are suggestive of a true outbreak[64,83] but in general do not fully meet diagnostic criteria. On a hypothetical basis, the early acquisition of immunity to infectious mononucleosis by mild and inapparent infections with EBV in childhood and the probable route of transmission via intimate oral contact in young adults weigh heavily against the occurrence of "epidemic infectious mononucleosis."

The high prevalence rates of EBV antibody in children in developing countries,[42] in nurseries,[124] and in orphanages[147] suggest that EBV spreads effectively in young children under circumstances of crowding and poor hygiene to reach almost all susceptibles. However, the contagious-

Table 3. EBV Infection Rates During Freshman Year and Percent Clinically Expressed in Different Colleges

Place	Number in study	Percent susceptible	Infection rate in susceptibles (%)	Percent clinical infectious mononucleosis
U.S. Military Academy[65]	1401	36	12.3	27.7
Five English schools[148]	1487	43	12.0	59.1
Yale University[132]	355	49	13.1	74.0

ness of infectious mononucleosis has been notoriously low in young adult populations; secondary cases have been rare in roommates of index cases,[37,65,79] in college dormitories,[37,45,132] and aboard ship.[122] The low contagiousness in college populations has been confirmed in recent studies employing the status of EBV antibody as a marker of susceptibility and of infection. Among susceptible and exposed roommates of Yale freshmen with infectious mononucleosis, no evidence of increased risk was found over the susceptible population as a whole[132]; however, there tended to be some aggregation of cases in social clusters in dormitories. In a more critical analysis of this issue at the U.S. Military Academy over a period of 4 yr, no evidence of increased spread of EBV infection to susceptible roommates exposed to an index case was detected as compared with susceptible roommates not so exposed.[65] Transmission of infection did not occur in a Polaris submarine in which two cases of infectious mononucleosis occurred.[142] In a family setting, about 10% of exposed and susceptible members will develop EBV infection.[71,85,151]

This low level of contagiousness of EBV infection in older children and in young adults of the same sex may be related to a high level of existing immunity and to the need for intimate oral contact. The rate of infection among susceptible persons who are known to have had intimate oral contact with patients having infectious mononucleosis or with established pharyngeal carriers of EBV has not yet been defined; it may well be high.

5.3. Geographic Distribution

Infection with EBV is world wide. Antibody to EBV has been demonstrated in every population thus far tested, including very isolated tribes in Brazil,[11] Alaska,[147] and other remote areas[57] where measles and influenza antibody are often lacking. Infection occurs earlier in life in developing countries.

Clinical infectious mononucleosis occurs in those hygienic and socioeconomic areas where exposure to and infection with EBV are delayed until older childhood and young adult life. This includes Australia, Canada, England, many European countries, New Zealand, Scandinavian countries, and the United States.[42] In contrast, at the University of the Philippines not a single case was recorded among 5000 admissions to the college infirmary, where laboratory facilities existed[47]; EBV antibody determinations in this college population revealed a very high level of prior immunity. In a recent study, the prevalence of EBV antibody had been found to vary in young adults entering the U.S. Military Academy from different areas of the United States.[65] The highest rate of 81.5% was found in cadets resident for 6 yr or more in the East South Central States and the lowest prevalence rate of 51.9% in the West North Central States. As admission to the Academy is based on competitive academic, athletic, and achievement values rather than on any social or economic considerations, a broad range of backgrounds would be expected.

5.4. Temporal Distribution

There is no clear-cut evidence of yearly fluctuations in the incidence of infectious mononucleosis, although appropriate morbidity data are not available to determine this accurately. Since 1948 in Connecticut, a yearly increase in incidence has been noted from 3.9 cases per 100,000 initially to 46.7 in 1967[21]; this probably reflects increased reporting rather than actual changes in incidence. In one Swedish hospital where the same population was served and the same diagnostic criteria were presumably applied over a period from 1940 to 1957, the hospitalization rate increased from 12 cases per year in 1940–1942 to 110 per year in 1955–1957.[144] Caution must be observed in interpreting hospital data in which there is no defined denominator. No changes in the incidence rates of infectious mononucleosis were noted at Yale University over a 5-yr period,[50] or in a careful study with a defined population base in Rochester, Minnesota, over the period 1950–1969.[69]

Earlier studies of college students at the U.S. Military Academy[82] and at the University of Wisconsin[37] showed a peak in February, some 4–6 wk after Christmas vacation, presumably due to increased exposure at these times. However, no clear-cut seasonal pattern has been seen in the CDC Surveillance Reports from 19 colleges and universities.[18] In a recent community study in Atlanta, Georgia,[68] two peaks were found, one

in early fall and a larger one in later winter and early spring; but in Rochester, Minnesota,[69] no seasonal peak was observed.

5.5. Age

The acquisition of EBV antibody by age is shown in Fig. 3 for three different geographic areas. Antibody occurs early in life in economically underdeveloped countries, often reaching close to 100% immunity by age 10. In contrast, clinical infectious mononucleosis is clearly a disease of older children and young adults in economically developed countries, with its highest incidence in the 15- to 25-yr-old age group. This has been true of data based on hospitalized cases in the United States,[55,68,69,95,110] France,[138] and Denmark,[146] on heterophil-positive cases identified in state public health laboratories,[25,38,109] and on recent community surveys in which the population at risk can be defined.[68,69] In results from the Atlanta community survey based on 575 heterophil-positive cases, the highest rate of 345.2 per 100,000 occurred in the 15–19 age group and the next highest, 122.8, in the 20–24 age group; 27 heterophil-positive cases occurred in the 5–9 age group, and four in the 0–4 age group. Figure 4 shows the distribution of cases in this study. A similar age distribution was observed in the Wisconsin State laboratory data based on elevated antibody titers in sera from suspected cases sent in for heterophil testing.[38] The peak frequency was in the 20–24 age group, in which 29.6% of the sera were positive. Some cases occurred at the extremes of age: 11.9% of the sera from suspected cases were positive in the 5–9 age group and 5.8% in the 65–69 age group.

The age pattern described is that of developed countries. In developing countries, the age distribution will be shifted downward because of the small number of children escaping infection until an age when the host response is that of recognizable infectious mononucleosis. In São Paulo, Brazil, for example, examination of records of the public health laboratory and several hospitals revealed only three fully confirmed cases, all in patients under the age of 10 yr.[48] In this setting, over 90% of the population have EBV antibody by age 10.[17]

5.6. Sex

No difference in EBV antibody prevalence rates by sex have been noted in population surveys.

Infectious mononucleosis occurs equally in both sexes, although girls appear to develop the disease earlier than boys,[68,69] with a peak occurring in girls at age 16 and boys at age 18 (see Fig. 4).

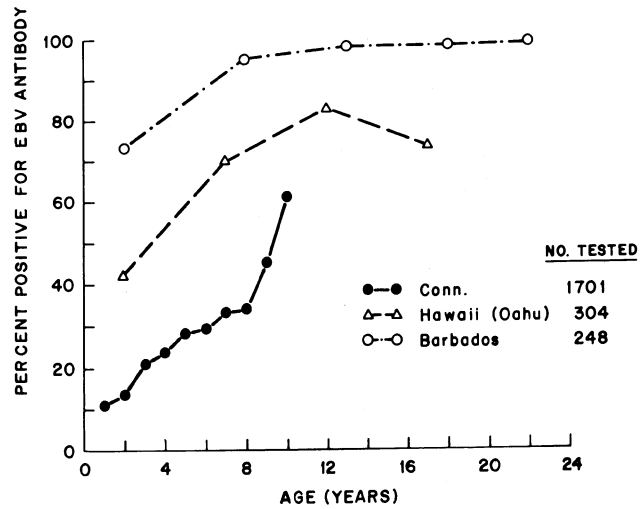

Fig. 3. Acquisition of EBV antibody by age in 3 different areas. Data derived in part from refs. 42 and 84.

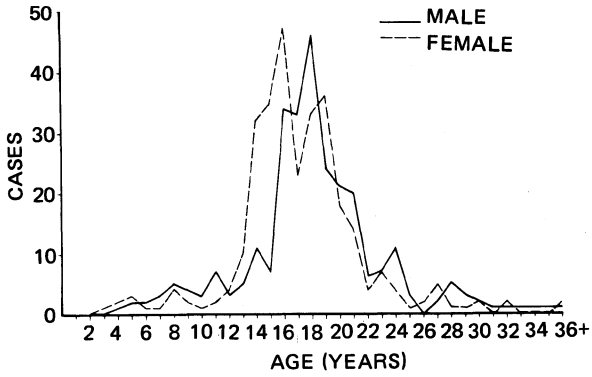

Fig. 4. Cases of infectious mononucleosis, by age and sex, metropolitan Atlanta, Georgia, 1968.

5.7. Race

EBV *infection* occurs in all ethnic groups; no evidence of differential susceptibility has been found.

Infectious mononucleosis in developed countries has been rare in blacks, but this probably reflects socioeconomic levels and earlier acquisition of infection rather than any difference in susceptibility. The incidence of the disease in whites in Atlanta, Georgia, was 30 times higher than in blacks.[68] Antibody prevalence to EBV among entering black cadets at the U.S. Military Academy was 85% as compared with 65% among whites.[65] In an analysis of prevalence rates among different ethnic groups in Hawaii,[84] higher rates were observed in Hawaiians and Filipinos than in Caucasians of the same age. However, socioeconomic levels, hygienic habits, and varying cultural practices in the home cannot be separated from the ethnic backgrounds.

5.8. Occupation

Infectious mononucleosis is a disease of the college student and of the white-collar worker,[55,110] especially young doctors and nurses. It is these groups that are apt to escape infection until young adult life because of a higher socioeconomic level and/or hygienic standards.

5.9. Occurrence in Different Settings

Pfeiffer's[126] original cases suggested that the disease had a familial pattern, and this has been partially borne out by subsequent studies. Analyses of sera from the Cleveland family study[27,71] produced evidence of several cases in three of seven families. There appeared to be a paucity of EBV infections in the 6–12 yr age group in this setting, with higher rates observed in children under 6 and over 12. Among 75 Canadian families, Joncas and Mitnyan[85] identified 67 persons lacking EBV antibody; in follow-up over approximately 2 yr, only 10.5% of these susceptible persons developed EBV antibody. In Sweden, Wahren *et al.*[151] found EBV antibody increases in seven of 21 members exposed to an index case; six of the 21 contacts lacked EBV antibody initially and three of these seroconverted.

The high rates of infectious mononucleosis in college and military settings have already been noted. However, during the early recruit training period in the armed forces infectious mononucleosis is not a common disease, unlike adenovirus, *Mycoplasma pneumoniae*, and other respiratory infections. This is probably due both to a high level of preexisting immunity among recruits and to the long incubation period of infectious mononucleosis, so that cases usually develop after the end of the usual training period and after dispersal of recruits to other military assignments. The former point is supported by the finding of an antibody prevalence rate of 85% in entering marine recruits at Parris Island,[94] and of 93% in army recruits at Fort Jackson, South Carolina.[51] Among the marine recruits at Parris Island whose sera lacked antibody, the infection rate was 18.5 per 1000 over the 16-wk training period; in those returning from a 13-month overseas assignment, the EBV infec-

tion rate was estimated at 23.8 per 1000. Among the 34 Fort Jackson recruits lacking EBV antibody, three recruits developed EBV antibody during the 16 wk of basic and advanced training,[51] a rate of 88 per 1000 recruits.

5.10. Socioeconomic Factors

Socioeconomic settings influence the incidence of both EBV infection and infectious mononucleosis but in opposite directions. Low socioeconomic groups have high rates of EBV infection early in life but little clinical infectious mononucleosis; high socioeconomic groups have low levels of EBV infection early in life but a high rate of clinical disease which occurs in the 15- to 25-yr-old group. Two examples illustrate their effect on infection rates. At the U.S. Military Academy at West Point, the EBV antibody prevalence rate was 77.1% in cadets coming from families earning under $6000 and only 58.6% among those from families with incomes of over $30,000.[65] In New Haven, the antibody prevalence among first graders in three schools serving a low socioeconomic group was 84.8% and in three schools serving a high socioeconomic group it was 37.8%.[136] A second serum sample collected from these same children 4–5 yr later revealed an EBV seroconversion rate of 50% among susceptible children from lower socioeconomic areas and only 2.4% in susceptible children in the higher socioeconomic group.

Table 4. Recovery of EBV from Oropharyngeal Excretions of 32 Patients with Infectious Mononucleosis

Days after onset	Throat washings		
	Number tested	Number positive	Percent positive
0–6	5	1	20.0
7–14	20	15	75.0
15–21	8	5	62.5
22–28	10	6	60.0
29–60	13	8	61.5
61–150	12	11	91.7
>150	19	6	31.6
Total	87	52	59.7

5.11. Other Factors

Little is known of the role of nutritional and genetic factors in relation either to EBV infections or to infectious mononucleosis. However, it is recognized that ABO blood groups are not correlated with susceptibility to infection or to clinical disease.[65,132] The relation to HL-A antigens has not been critically explored. It seems possible that genetic control of the immune response might play a role in the severity of clinical illness, in the persistence of virus, and in possible oncogenic sequelae.

6. Mechanism and Route of Transmission

The major route of transmission of infectious mononucleosis in young adults is probably through intimate oral contact in kissing with the exchange of saliva and perhaps of infected cells, as first suggested by Hoagland in 1955.[79] This concept is supported by three types of circumstantial evidence. First, close personal contact without kissing as in roommates of infected patients[132] or in such confining environments as a destroyer[122] or a Polaris submarine rarely leads to secondary cases[142]; this has been true even when the exposed roommate is known to lack EBV antibody and is followed closely over 2 months for the appearance of antibody or of clinical symptoms.[65] Second, a history of intimate oral contact within the appropriate incubation period is common in young adults developing infectious mononucleosis[79] and occurs statistically more frequently than in healthy controls or patients with acute respiratory infections.[37] Third, the presence of EBV has been demonstrated in the pharynx during acute illness and during convalescence for periods of many months (Table 4).[19,59,103] In addition, cross-sectional studies of presumably healthy adults also have shown EBV pharyngeal excretion in 15–20% of young adults.[59,143] One investigation found a leukocyte transforming factor, presumably EBV, in the throats of 18% of 368 patients attending an outpatient clinic.[20] Transmission of EBV infection may also occur via the transfusions, usually without illness.[58,75,150]

This prolonged carrier state in persons lacking EBV antibody after clinical infectious mononucleo-

sis and presumably also following inapparent EBV infection may serve as the principal source of exposure in young adults. The long duration of virus excretion may explain the difficulties in tracing transmission of disease from case to case. Virus excretion occurs in the presence of circulating antibody, which suggests that humoral antibody does not have a major role in the regulation of oropharyngeal shedding. Clearly, identification of the specific oropharyngeal cells which produce infectious virus remains an important area for further investigation.

The mechanism of transmission accounting for the rapid and high rate of acquisition of EBV antibody in nurseries and in young children in low socioeconomic circumstances[124,147] is not definitely known. Presumably transfer of infected saliva on fingers, toys, and other inanimate objects in settings of poor hygiene can account for much of the spread of infection. Perhaps more cell-free virus is released in childhood infections. It is not known if EBV occurs in urine or feces and could be important epidemiologically.

From a practical standpoint, the low contagiousness of the disease in young adults eliminates the need for strict isolation procedures.

7. Pathogenesis and Immunity

The incubation period of infectious mononucleosis is 4–7 wk[37,81] in college students. This estimate is based on well-defined, often single contacts between an index case and a member of the opposite sex involving intimate oral contact.

Studies of the recovery of EBV from oropharyngeal secretions of patients with infectious mononucleosis have revealed that virus shedding occurs during the acute illness and from several weeks to many months after onset of the syndrome, but the type of cells involved is unknown. Transformation of umbilical cord leukocytes into continuous cell lines has been the assay system used for demonstration of the virus, and this transformation has been neutralized by sera containing EBV antibody, but not affected by sera lacking this antibody.[103] In addition, transformed leukocytes acquire EBV genome, demonstrated by nucleic acid hybridization, and express EBV-associated antigens. The virus has been detected in throat washings for prolonged periods and as indicated in Table 4 is regularly recovered several months after clinical illness. In six of 19 patients, the agent was still present over 5 months after disease had occurred and in one case was detected 16 months after onset. No special clinical characteristics have as yet been identified in those cases associated with prolonged oropharyngeal virus shedding.

Prior to the onset of definite symptoms in young adults, there is frequently a history of ill-defined complaints, such as malaise and easy fatigue. It has been suggested that an early, abortive infection of this type may occur in children without subsequent development of classical infectious mononucleosis.[37] In an analysis of 100 presumed heterophil-negative cases of infectious mononucleosis involving the 0–9 yr age group studied in England,[83] the incubation period was shorter than for adults and estimated at 4–10 days.

The pathogenesis of infectious mononucleosis is intriguing not only because the self-limited clinical disease is manifested primarily in young adults but also because of the suspected oncogenicity of the virus and its relationship to African Burkitt lymphoma and to nasopharyngeal carcinoma. An understanding of what immunological mechanisms turn infectious mononucleosis on and what turn it off is important in this context.

Transient depression of delayed hypersensitivity has been described during acute infectious mononucleosis,[10,62] and depressed T-cell stimulation[134] by phytohemagglutinin has been recorded. Recently, profound alterations in cell-mediated immunity were demonstrated by intradermal skin tests, *in vitro* lymphocyte stimulation, and enumeration of absolute numbers of peripheral blood T and B cells.[97] Lymphocyte responsiveness to a variety of mitogens and antigens was found to be depressed during the first weeks of illness. Serial studies of the interaction of T- and B-cell populations during acute disease indicated that peripheral blood B cells increase during the first week of illness and return to normal levels several weeks later. In contrast, T cells reach peak values during the second week of disease and remain elevated for approximately 5 wk.[97]

Recent investigations indicate that both T and B cells may be transformed into atypical lymphocytes characteristic of this disease.[31,60,97] These observations suggest that B cells may be trans-

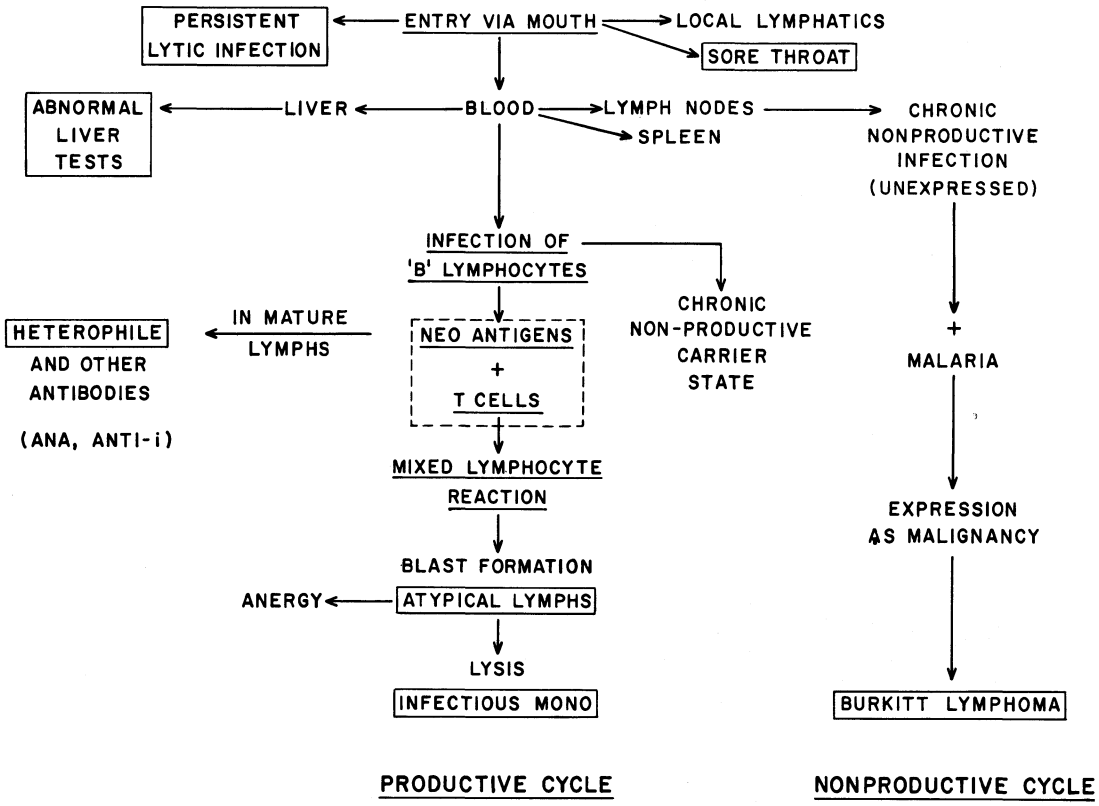

Fig. 5. Hypothetical pathogenesis of EBV infection.

formed by infection with EBV and T cells may be transformed as an immunological response to the viral antigen itself or to altered antigens on the surface of the B cells.

A version of pathogenesis incorporating our current understanding of the role of EBV is presented in Fig. 5.[44] EBV appears to enter the oropharynx in young adults, and multiplies locally, producing a lytic and persistent infection.[32] It then enters the bloodstream and possibly the gut, although the latter has not yet been established. Lymphocytes of the B type are infected and a long-term carrier state is established; some of these transformed B cells contribute to the atypical lymphocytosis early in disease.[60,97] There may also be an immunological response of T cells to B cells whose membrane has been altered by an EBV-induced antigen. This results in T-cell proliferation,[150] transformation, and a major outpouring of atypical lymphocytes with T-cell characteristics. In support of the presence of a new membrane antigen, lymphocytes from patients with acute infectious mononucleosis have been found to cause stimulation of convalescent leukocytes from the same persons.[7,87] During the acute illness, the activated T cells may cause the destruction of EBV-infected B cells, as has been shown *in vitro* and in other viral systems.[67,97,140] This destruction may account for the inability to demonstrate EBV or its genome in *fresh* lymphocytes from patients with acute infectious mononucleosis. That not all B lymphocytes are destroyed is shown by the presence of a sufficient number of infected cells to initiate a long-term culture of EBV-infected B lymphocytes, perhaps through secondary infection of other B cells *in vitro* when immunological inhibitors in the serum are removed.[32] The mechanism of heterophil produc-

tion is still unexplained, but knowledge that its appearance is most common in EBV infections of young adults[42] and that the degree of expression and release of EBV *in vitro* varies in lymphocytes from donors of different ages suggests avenues of investigation. For example, EBV-infected fetal lymphocytes do not have demonstrable VCA antigen but do contain EBV nuclear antigen by the EBNA test[101,129] and complement-fixing antigen.[101,104] In lymphocytes from adults and from marmosets, EBV may mature more fully, resulting in release of EBV antigens to other lymphocytes. Heterophil antibody may occur in response to membrane-induced antigens of EBV expressing themselves more fully in lymphocytes of young adults than in those of younger children or in fetal lymphocytes.

While the presence of antibody to the viral capsid antigen of EBV has been shown to indicate protection against infectious mononucleosis and its absence indicates susceptibility,[44,50,65,113,132] the actual antibody providing immunity is probably the neutralizing antibody for which tests have recently been developed.[78,105] One attack of infectious mononucleosis confers a high degree of durable immunity to subsequent attacks of clinical infectious mononucleosis.[16,50,105] Presumably subclinical or inapparent EBV infections also confer lasting immunity. One fairly well-documented case of clinical recurrence has been reported[9] and a resurgent anamnestic heterophil response after infectious mononucleosis has also been noted in patients subsequently developing a respiratory infection.[8,80] Reinfection with or without clinical illness has not yet been fully documented by appropriate heterophil and EBV antibody tests.

8. Patterns of Host Response

8.1. Clinical Features

When infection with EBV occurs in childhood, a mild, nonspecific illness or an inapparent infection may develop, both of which are associated with the appearance and persistence of antibody to EBV. If exposure and primary infection are delayed until adolescence or young adulthood, the characteristic clinical picture usually occurs. This consists of fever, pharyngitis, and cervical lymphadenopathy, accompanied by splenomegaly in 50% and hepatomegaly in 10%. The pharyngitis is often associated with a whitish or a gray/green exudate having an offensive odor. The eyelids may be swollen, and petechiae occur on the hard palate in 25% of cases.

Abnormalities of liver function tests are a regular feature of infectious mononucleosis, and clinically recognizable jaundice occurs in 5% of cases. Rarer manifestations include a variety of central nervous system syndromes (encephalitis, meningoencephalitis, Guillain-Barré syndrome), pneumonitis and pneumonia, thrombocytopenic purpura, myocarditis, and nephritis.[39,82] The major complications include splenic rupture and airway obstruction from exudative pharyngotonsillitis. About 50 deaths have been reported due mostly to central respiratory failure. An immunological deficit, especially in cell-mediated immunity, may be involved, as suggested by the recent report of two deaths in a family.[5]

The frequency with which EBV infections are expressed as clinical illness in young adults has varied in different populations. In a recent study of a cohort of U.S. Military Academy cadets over a 4-yr period, only 26.4% of 201 infected with this agent developed heterophil-positive clinical infectious mononucleosis.[65] The apparent:inapparent EBV infection ratio in different years ranged from 1:1 to 1:2.6 in this population. Comparison of the frequency of clinically expressed infectious mononucleosis in freshman students in three different settings is presented in Table 3. The reasons for the differences are not known but may relate to the motivation to seek medical care, physical fitness, or concern about the effect of hospitalization on academic and school activities.

The relationships between clinical features and antibody levels in a typical heterophile-positive case in an 18-year-old student are shown in Fig. 6. Following a prodromal period associated with fatigue, fever, and headache over several days, the onset of sore throat, cervical adenopathy, and recurrent fever developed during the second week. Characteristic blood changes were present on the third day after onset, and the patient's lymphocytes contained EB virus-associated complement-fixing antigens (EBNA) in a nuclear location. The heterophile antibody titer was negative on the first day of symptoms, rose to 1:14 after guinea pig absorption

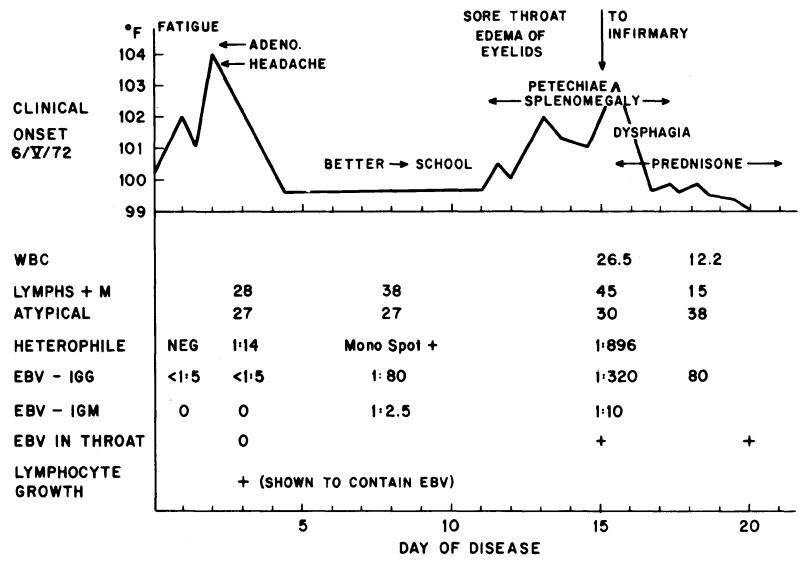

Fig. 6. The relation between clinical and laboratory features of infectious mononucleosis in an 18-year-old male.

two days later, and then increased to 1:896 on the 15th day. In contrast, Epstein-Barr VCA antibodies of IgG type, undetectable on the third day of illness, were present on the 8th day and rose to a level of 1:320 by the second week. EBV-specific IgM antibodies were demonstrable on the 8th day at a titer of 1:2.5, which then increased to 1:10 by the 15th day.

No direct correlation has been found between the levels of Epstein-Barr VCA and heterophil antibodies, nor between VCA, early antigen, and EBNA antibody levels and the severity of clinical symptoms and hematological changes.[73,77,114]

8.2. Diagnosis

The diagnosis of infectious mononucleosis is based on a typical clinical picture with the triad of fever, sore throat, and cervical lymphadenopathy, the occurrence of at least 50% lymphocytosis with at least 10% atypical lymphocytes, and the appearance of heterophil antibodies. The presence of antibodies to EBV is an absolute requirement in doubtful or heterophil-negative cases. Antibody to viral capsid antigen is usually present at the time the physician first sees the patient, and only 15–20% of patients will show a subsequent rise in titer; antibodies to early antigen appear later but are present in only 75% of typical cases.[77] Antibodies to EBV-associated nuclear antigen (EBNA) usually arise 1 month or more after illness and probably persist for life.[73] EBV-specific IgM antibodies are demonstrable in 85% of cases during acute illness. Figure 7 depicts the course of EBV-specific IgG and IgM antibodies during the course of illness. IgG antibodies persist for years, perhaps for life; IgM antibodies usually disappear in 3–6 months. At present, there is no practical and rapid EBV antibody test for the diagnosis of infectious mononucleosis.

The main reliance in diagnosis must be placed on the heterophil antibodies which are of the IgM type. Methods in most common use are the sheep and horse cell agglutination tests after absorption of the serum with guinea pig kidney to remove Forssman antibody, and the beef cell hemolysin test, which does not require absorption. The appearance and persistence of these tests during acute and convalescent infectious mononucleosis are shown in Fig. 8. The beef hemolysin test is the most specific but has a short duration; the horse cell test is the most sensitive and most persistent, with positive tests present for a year or more in 75%.[52] The appearance and persistence of antibody to horse cells have been found to follow mild and subclinical episodes of infectious mononucleosis provided that sera are collected over a long enough time. This test may be useful in childhood infec-

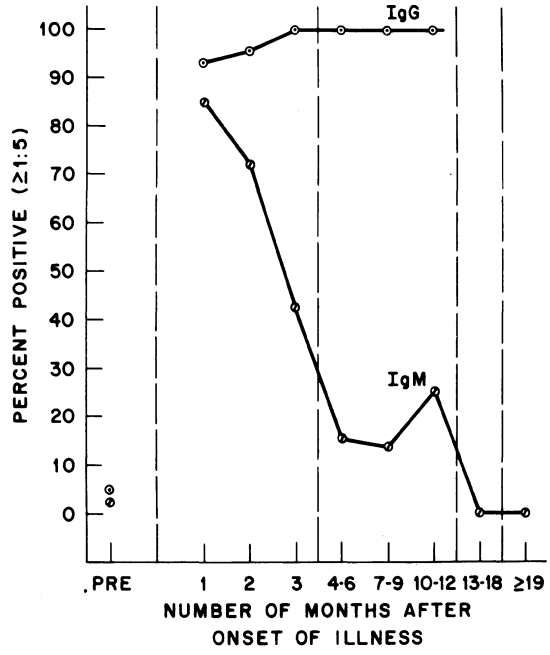

Fig. 7. Appearance and duration of IgG and IgM antibodies specific for Epstein-Barr virus during infectious mononucleosis. From ref. (52).

tions with EBV, which are often heterophil negative by other tests or when inadequately followed.

Development of EBV antibody has also been shown in cases where the clinical and hematological characteristics are those of infectious mononucleosis but the heterophil antibody remains persistently negative.[50,114a] These heterophil-negative, EBV antibody positive cases appear to be common in infants and children, but are rare in adults. Infection with cytomegalovirus (CMV) may also produce a clinical picture of heterophil-negative mononucleosis that is hard to distinguish from classical infectious mononucleosis; however, it usually occurs at a later age and adenopathy and exudative pharyngitis are rare.[91]

9. Control and Prevention

Attempts to control infectious mononucleosis and EBV infections by interrupting the presumed chain of transmission seem neither realistic nor perhaps desirable in light of our current knowledge. If intimate oral contact represents the major route of spread in young adults, there seems little likelihood of interdicting this practice or of preventing salivary transfer. If poor hygienic conditions promote the spread of EBV infections in young children, then improvement in hygienic and socioeconomic circumstances might reduce their incidence. Unfortunately, control of spread at this time when infection is largely mild and asymptomatic might simply delay exposure to later childhood and young adult life when the majority of EBV infections are expressed as clinical infectious mononucleosis.

The high degree of protection against infectious mononucleosis provided by natural infection with EBV suggests that a vaccine capable of evoking similar humoral, cell-mediated, and local immunity might be highly effective. An attenuated live vaccine administered orally would be the most desirable. However, the apparent limitation of viral multiplication *in vitro* to primate lymphocyte suspension cultures and the low yield of infectious

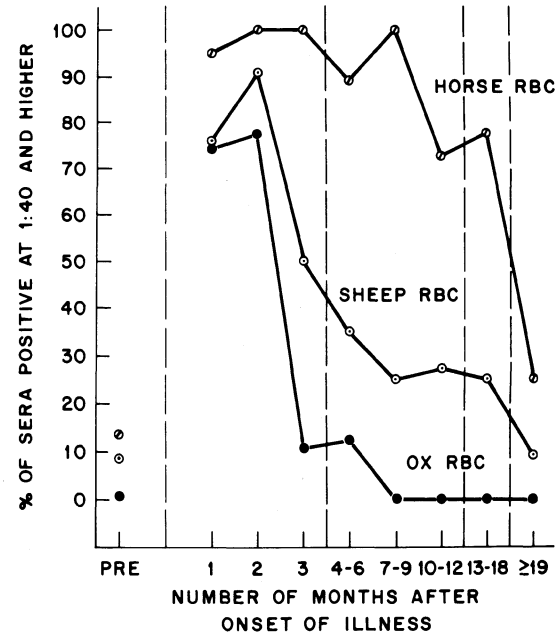

Fig. 8. Persistence of heterophile antibodies during infectious mononucleosis. RBC = red blood cells. From ref. (52).

virus released are formidable technical obstacles at present.

If an effective vaccine were available, it might best be given on entrance to high school to permit natural infection with little clinical illness to occur before that and then to prevent clinical illness in the young adult. It would be useful only in developed countries with a high incidence of infectious mononucleosis.

The oncogenic and transforming potential of EBV poses a serious question of risk for use of a vaccine. The numerous problems associated with long-term surveillance for possible complications would be considerable, but these risks in developed countries may not be great. The association between EBV and cancer is evident primarily in Burkitt lymphoma in Africa and in nasopharyngeal carcinoma (NPC) patients of Chinese descent in the Far East. In these settings, clinical infectious mononucleosis is too rare a disease to merit vaccination. Furthermore, tumor development is associated with malaria in African Burkitt lymphoma and immunogenetic susceptibility to NPC in Chinese. In the absence of malaria and genetic susceptibility, as in the United States, both of these tumors are very rare. Such considerations suggest that if the technical problems of viral attenuation and vaccine production can be overcome, oncogenicity would be a hazard of negligible magnitude in the United States and other countries where clinical infectious mononucleosis is a common and disabling disease.

10. Unsolved Problems

The problems that remained to be solved concerning the nature of EBV infections are summarized in Table 5. While EBV is well established as the cause of heterophil-positive infectious mononucleosis and "turns on" the immunological events that are involved in the pathogenesis of the clinical disease, we have little information as to what "turns off" the lymphoproliferative cycle and makes the disease a benign, self-limited one.

The persistence of EBV in circulating lymphocytes following infection and the presence of a small number of "atypical lymphocytes" in healthy persons suggest that the proliferative process is not fully "turned off" but held under careful control and immunological surveillance; the continued presence of anti-VCA, anti-EBNA, complement-fixing, and other viral antibodies supports the concept of continued antigenic stimulation. Viral excretion in the pharynx certainly continues long after infection, perhaps intermittently for life, but the cells supporting this multiplication are presently unknown. There is also the existing paradox that neither EBV nor viral genome has been demonstrated in fresh lymphocytes during infectious mononucleosis except in overwhelming and fatal infections; whether this is due to extremely small numbers of infected cells or represents viral multiplication in secluded cells outside the circulation is unknown (see footnote on p. 215). The source of the heterophil antibody and the variety of other non-viral antibodies that appear during the course of infectious mononucleosis remains a mystery; whether these antibodies appear in milder EBV infections in smaller amounts and at a later time is not known, but very recent evidence suggests this may be so. A simple EBV-specific antibody test is needed in the diagnostic laboratory to confirm presumably heterophil-negative cases. An explanation is lacking for the observation that a more severe

Table 5. Unsolved Questions in EBV

1.	What turns infectious mononucleosis off and makes it a self-limited disease?
2.	What cell supports pharyngeal multiplication of EBV?
3.	Why can't EBV be demonstrated in lymphocytes in acute infectious mononucleosis?
4.	Where does heterophil antibody originate?
5.	Can a practical EBV antibody test be developed for diagnostic use?
6.	What causes different responses to EBV at different ages?
7.	Is an infectious mononucleosis vaccine possible?
8.	Does EBV cause cancer?
9.	Why are EBV antibody titers high in some chronic diseases?
10.	What accounts for the geographic distribution of Burkitt lymphoma and nasopharyngeal cancer?

clinical response to EBV infection occurs when exposure is delayed until older childhood and young adult life, similar to the more frequent occurrence of jaundice and paralysis in hepatitis and poliomyelitis infections in adults. If the clinical syndrome of infectious mononucleosis is primarily an immunological response of T cells to EBV-induced neoantigens on the B-cell membrane, then one can speculate that induction of membrane antigens occurs more commonly in lymphocytes from mature individuals in which more complete virus is formed than in lymphocytes from young children; this idea is supported by the failure of EBV-infected cord cells to produce viral capsid or membrane antigens. The possibility of a vaccine has already been discussed, and the relation of EBV to cancer is explored in other chapters of this book. Infectious mononucleosis continues to be an important model for studying the immunological and virological events involved in a persistent and possibly neoplastic infection.

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12. Suggested Reading

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