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## Epidemiologic Studies Assessing the Role of the Epstein-Barr Virus in Hodgkin's Disease

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The hypothesis that an infection plays a role in the etiology of Hodgkin's disease (HD) is suggested by both its clinical and histologic features. Its bimodal age-incidence pattern also suggests an infectious process among younger persons. In economically advantaged populations, the first peak occurs among young adults, while in disadvantaged populations, it occurs among children at a much lower frequency. It appears that the age distribution of HD shadows that of susceptibility to common childhood infections, such as the Epstein-Barr virus (EBV); furthermore, that risk of HD is increased among those susceptible to a relatively late infection, in parallel with infectious mononucleosis (IM), and it has been found that people who have had IM have about three times the expected rate of HD. Serologically, there is a consistent association between EBV and HD. As a group, patients have an altered antibody pattern against EBV which suggests chronic reactivation, both following and preceding diagnosis. This altered pattern is common to all age groups. Severity of infection may alter host control among younger people, while diminished cellular immunity with aging may allow similar reactivation among older persons. Whether the EBV plays a direct role or simply reflects the action of a more primary factor is unknown.

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The hypothesis that the pathogenesis of Hodgkin's disease (HD) involves an infectious agent has been considered since it was first described by Thomas Hodgkins in 1832 [1-2]. This is suggested primarily by its clinical features of night sweats, fever, and lymphadenopathy, as well as histologic features resembling an inflammatory granuloma. Historically, the classification of HD has been a matter of contention. The frequent coexistence of active tuberculosis was taken as evidence of its infectious nature [1]. Evidence of its malignant nature include metastatic spread, transplantability, and the aneuploidy and clonality of the diagnostic Reed-Sternberg cell [3]. An early proponent of the Epstein-Barr virus (EBV) as a candidate agent was Dr. Alfred Evans [4], and it has been my privilege to collaborate with him for nearly 15 years in assessing the role of EBV in the etiology of HD. This paper reviews Dr. Evans's contribution in this research.

HD is a relatively rare disease, the age-adjusted incidence (per 100,000 person-years) being 2.9 for white Americans and 1.6 for blacks. Generally, rates are higher among males. Because of its unusual age distribution, the majority of diagnoses occur before age 45 [5]. Recent advances in treatment have dramatically improved survival rates, so that current expectation for five-year survival is 74 percent, nearly twice that of the early 1960s [6].

321

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The epidemiologic features of HD also suggest an infectious etiology in several ways. Most malignancies have an age incidence curve which exponentially increases in the last decades of life. In contrast, HD has a bimodal age-incidence curve with an initial peak occurring in young adulthood in industrialized populations, and the second among the oldest persons (Fig. 1). In economically developing populations, however, the first peak occurs in childhood, almost exclusively among boys, and little disease is diagnosed among young adults. Transition populations have intermediate patterns [7].

As first suggested by Newell in 1970, this geographic variation among younger people in relation to economic conditions is quite reminiscent of the epidemiology of paralytic polio (PP) in the pre-vaccine era, in which risk of disease with infection was greater among adolescents and adults than among children [8]. In the polio model, socioeconomic conditions were associated with the age-incidence of PP, as these affected the age at which people were infected with these widespread viruses. Age at infection, in turn, determined the likelihood of developing neurologic complications. Most infections produced no or inapparent disease and resulted in life-long immunity. Paralysis was generally rare with infection in preschool children. At older ages, the probability increased directly with age. Thus, in developing countries, most PP was seen among infants because essentially all were infected quite early in life. In economically advantaged populations, PP was seen among older children and young adults, reflecting an increase in susceptibility to later infection because of better sanitary conditions and less crowding. For both HD and PP, age-incidence patterns vary geographically with level of economic development. For both, disease risk is greatest among young children from the least favorable environment but greatest among adolescents and young adults from the most favorable. For both, a temporal upward shift in the age-incidence pattern has accompanied economic development. Analogy suggests that HD among young adults may also be related to the effect of delayed age at infection with a common childhood virus [9]. The reported associations of risk of HD among young adults with risk factors associated with higher social class support this hypothesis [10,11].

The bimodality of the age-incidence curve of HD as well as the observation that, in contrast to younger persons, there was little geographic or temporal variation in the incidence among older persons, suggested to MacMahon in 1957 that there may be separate etiologies operating in the two age groups. He noted that the relative proportion of males was higher among older persons and that being Jewish was a risk factor among older people, but not apparently so among young adults [12].

Evidence that young and older patients differed clinically also supports the two-disease hypothesis. In terms of the histologic sub-types, the nodular sclerotic form predominates among younger people and the mixed-cellularity form among older [13]. Eosinophilic infiltrates are much more common in the young, while atypical mitoses and other features of malignancy are more common in the old [14]. Older patients are more likely to have abdominal lesions and less likely to have mediastinal involvement [15,16]. However, others have interpreted these features as reflecting age- and sex-associated host response to a common neoplastic process [17].

The epidemiologic features of HD among young adults were also reminiscent of that of infectious mononucleosis (IM), a disease for which Dr. Evans was instrumental in proving the role of delayed EBV infection, as both are diseases of young adults in economically advantaged populations [18]. The similarities prompted Dr. Evans to

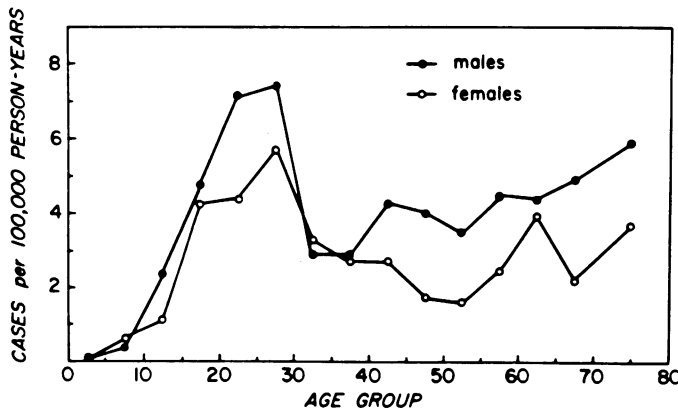


FIG. 1. Age- and sex-specific incidence rates of Hodgkin's disease in the Boston and Worcester Massachusetts Standard Metropolitan Statistical Areas from July 1973 through 1977. Used with permission of the *New England Journal of Medicine* [44].

propose in 1971, "Perhaps some cases of HD represent either the host's response to EBV infection that is delayed until the early twenties or a secondary response in persons with a genetic susceptibility or an impaired immunologic system, or both" [4].

The association between HD and EBV is supported by two sets of findings. The first concerns the consistent finding of increased risk of subsequent diagnosis of HD among persons who have been diagnosed with IM. In six published studies which followed over 41,000 individuals with a serologic diagnosis of IM, a total of 37 HD cases were observed, with 12 expected [19-24].

The second set of findings concerns elevated titers of antibodies against the major determinants of the EBV. These include the viral capsid antigen (VCA); antibodies against the VCA are commonly measured as evidence of EBV infection. In addition, antibodies against the early antigen (EA) are present during primary infection and during reactivation. Two EA antibody components can be distinguished: the diffuse (D) which is elevated in nasopharyngeal carcinoma, and the restricted (R) which is elevated in Burkitt's lymphoma. A final determinant is the nuclear antigen (EBNA) which is a virus-induced antigen expressed in lymphocytes which carry the EBV genome, i.e., "transformed" B cells [25].

The most common finding was that HD patients as a group had elevated titers against the VCA in comparison to controls, who generally were patients with other diseases or blood donors [26-41]. Several studies also reported elevated titers against EA [29-30,34-28]. None of these studies were analyzed for confounding by the various antibodies nor for history of IM. Although several studies evaluated effect modification among the histologic sub-categories, no consistent variation was evident.

In the late 1970s, Dr. Evans evaluated the serologic association between the EBV and HD in two separate investigations conducted in Sao Paulo, Brazil. In the first, the antibody profiles of 67 cases were compared to that of 67 healthy controls, matched for age, sex, and socioeconomic status [42]. In this study, the patients had a significantly elevated titer distribution against the VCA as well as a significantly higher proportion with antibodies against the EA, indicative of virus activation. In addition, the subjects were compared for their antibody response to nine other common infections, and no differences were found. In a second study, Dr. Evans compared 70 cases to 161 of their relatives, each of whom was paired to a population control. Again the cases had elevated antibody patterns in comparison to both related and unrelated controls. In

addition, those relatives of cases with elevated antibody levels had significantly higher titers than those relatives of cases with lower EBV levels [43].

It was during the mid-1970s that our collaboration began in a large, population-based, case-control study of Hodgkin's disease conducted in the combined metropolitan areas of Boston and Worcester, Massachusetts. This study included 326 newly diagnosed cases aged 15 years or older, and two control groups. The first comprised 650 controls identified by a randomizing procedure from the population base with a comparable age and sex distribution to the cases. The purpose of this control group was for the analysis of interview information, with the aim of identifying risk factors which influence or reflect delayed susceptibility to common childhood infections. A second comparison included 315 siblings of cases. This control group was used for the serologic component of the study. The use of siblings eliminated differences in childhood social environment.

The details of the interview study have been published elsewhere [44,45]. In brief, we found that among young adults (15–39 years of age), risk of HD was associated with small family size and early birth order, high maternal education, and low density housing in childhood—all factors which would favor susceptibility to “late” infections. Similar risk factors held for middle-aged persons (40–54 years). Among older persons, however, risk was independent of these social class variables. The latter finding again suggests that the pathogenesis of HD among older persons is independent of that among younger persons.

The details of the serologic study have also been published [46]. These analyses involved the 304 cases and 285 siblings for whom blood specimens were obtained. Again we found that risk of HD was significantly associated with elevated titers of anti-VCA and both components of the EA. On multivariate analysis based on the matched data, risk among those with elevated VCA antibodies ( $\geq 1:320$ ) was more than four times that of persons with low titers ( $\leq 1:40$ ). For the EA, elevated titers against the D component were associated with a twofold risk. These findings held true for all age groups. The study also found a significant elevation of titers against the cytomegalovirus (CMV), but not consistently so among all age and sex groups.

The most interesting finding was an association of antibody level and history of IM among both cases and controls. Among cases, 35 reported a history of IM. Of these, 66 percent had elevated titers against VCA compared to 32 percent of the 227 cases who had no history. Similarly among the sibling controls, 17 percent of the 23 with a history of IM had elevated VCA antibodies compared to 12 percent of 227 sibling controls without. A parallel finding was true for elevated levels of antibodies against the EA-D; the results for cases were 46 percent and 27 percent, respectively, and for the siblings, 30 percent and 10 percent. It is important to note that essentially all these subjects had had an EBV infection. It is likely that those who reported having IM had had the infection on average somewhat later in life than those who had had an unrecognized EBV infection. Thus the finding supports the hypothesis that higher VCA antibodies are associated with a more severe EBV infection [47], which is associated with age at infection.

At this point it was clear that the serologic association between EBV and HD was valid, but whether it was itself primary, or simply secondary to the effects of treatment or to the immune dysfunction of the disease itself was not known. The only way to evaluate the latter explanation would be to determine the antibody status of a sample of cases during the latent period—that is, between infection with EBV and the diagnosis of HD. It was at this point that Dr. Evans's long-standing interest and

activity in the establishment of serum banks as the basis for follow-up studies related to infectious exposure provided the means to an end.

The first step was to do a pilot study. Dr. Evans contacted his long-time colleague, Dr. George Comstock of Johns Hopkins University. Dr. Comstock had established a serum bank in 1974 for 25,802 participants of the Washington County (Maryland) population study of determinants of cancer. This population had been prospectively kept under surveillance for subsequent diagnoses. Dr. Evans asked Dr. Comstock if there were any participants who had developed HD and, if so, to send their sera and that of matched controls to Yale for analysis. Three sets of case and control sera arrived. For two sets, the cases had clearly altered patterns of antibodies against EBV in blood drawn 11 and 21 months prior to diagnosis. In order to evaluate specificity, antibodies against three other herpesviruses—CMV, simplex, and varicella—were assayed; no differences were apparent. The third set was normal; it was from a case of histiocytic lymphoma [48].

Armed with the promise of the pilot study, a large, multi-center collaboration was organized to search for additional serum specimens which had been drawn and stored from people who later developed HD. Because of Dr. Evans's extensive network of colleagues, a collaboration was established which together held blood specimens from over 240,000 individuals for whom subsequent diagnoses of malignancy could be determined either from computerized medical records or by direct follow-up which was being done for other purposes. The details of this study will be published elsewhere. In summary, we found serum specimens stored prior to a diagnosis of HD of 43 persons. These were matched to specimens drawn at the same time from 96 controls of the same age and sex. For the cases, the specimens were obtained in the period 13 years to eight months prior to diagnosis. We found that the antibodies against VCA and the diffuse form of EA were significantly elevated among cases with a three- and fourfold risk, respectively. In addition, cases were distinguished by elevated antibodies against EBNA and by the presence of IgA antibodies against the VCA. The pattern seen in these pre-diagnosis specimens, like that in the post-diagnosis specimens, suggested chronic EBV replication.

Although other risk factors differ between age groups, altered EBV antibodies are common to all. Severity of infection may result in altered host control among younger people, while diminished cellular immunity with aging may allow similar reactivation among older people. The observation of HD occurring in relation to infection with the human immunodeficiency virus, which also allows a high level of EBV reactivation, would support this proposal [49,50].

Whether chronic EBV replication has a direct role in pathogenesis is not at all clear. The lack of evidence of virus DNA in involved tissue [51,52] argues against direct genetic damage by the virus. It is conceivable, however, that this chronic antigenic stimulation may lead to altered gene expression, resulting in the immune dysfunction and cellular abnormality of HD [53]. An alternative explanation is that the chronic EBV expression is secondary to another, more fundamental factor, such as another viral infection. Whatever the solution of this question, our understanding of the etiology of HD has been greatly enhanced by the contribution of Dr. Evans.

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