

# Hodgkin's Disease Following Infectious Mononucleosis

## A Case Report

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THE AETIOLOGY of Hodgkin's disease (HD) is still unknown. However, several epidemiological studies have postulated an association between HD and preceding infectious mononucleosis (IM) [1, 2]. The causative agent in IM is the Epstein-Barr virus (EBV) [3]. Although in many cases of HD serum titers against EBV are somewhat increased [4, 5], some cases of HD show no antibody evidence of EBV infection. Serious doubts as to any specific role for EBV in HD come from cases with established HD in which IM occurred during the course of the disease [6, 7].

The purpose of this communication is to show by DNA analysis of the tumor cells—searching for EBV genomes—that, although HD developed shortly after IM and antibody response to EBV was compatible with the convalescent phase of IM, EB-viral infection most likely may not be directly oncogenic but rather may contribute to an immunologic state which in turn predisposes to malignancy.

### CASE REPORT

In November 1979, a 21-yr-old male patient noticed a painless lymph node swelling of the right side of his neck with a concomitant swollen right tonsil. Otherwise he felt well and had no signs of malaise, weight loss or night sweats. About six weeks prior, he

remembered having symptoms of a common cold. On 21 December, a lymph node biopsy was done revealing an unspecific, chronic inflammation compatible with a viral infection. In January 1980, IM was suspected because of a slightly positive Paul-Bunnell test for heterophil antibodies (1:16) as well as positive titers of specific IgM and IgG antibodies (1:16 vs 1:64) against EBV viral capsid antigen (VCA). The WBC at that time was  $5 \times 10^9/l$  with a differential of 57% neutrophils, 5% eosinophils, 1% basophils, 10% monocytes and 27% lymphocytes. Three weeks later, the Paul-Bunnell test and EBV-VCA IgM antibodies had become negative, EBV-VCA IgG-(1:128), early antigen (EA)-(1:10) and Epstein-Barr virus nuclear antigen (EBNA)-antibody titers were positive. Within another three weeks EBV-EA antibodies became negative too.

Since the patient felt no improvement of his swellings, particularly of his right tonsil, he had the latter removed in the end of February. Histological examination showed an identical picture to that of the lymph node biopsy performed one month earlier. Therefore, no treatment was initiated. Because of the persistent cervical lymph node enlargement involving the right anterior triangle, the patient was transferred to our hospital. He was otherwise well and physical examination revealed no other abnormalities. X-ray of the chest and thoracic inlet were normal. Routine laboratory tests were within normal range (ESR, sodium, potassium, serum electrophoresis, transaminases, alkaline phosphatase, peripheral blood—except for 8% atypical mononuclear cells in the differential count). The IM slide screening test (Monospot) was ne-

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gative as were EBV-VCA IgM, EBV-EA and heterophil antibody titers. EBV-VCA IgG (1:256) and EBNA were still positive. Antibody titers to adeno-, cytomegaly- and mumps virus were negative.

Finally, on 13 April another large lymph node biopsy from the same region was performed, mainly in an attempt to search for EBV genomes in the persistent lymph node tumor. Employing several sensitive methods for detection of EBV-DNA sequences, no EB viral genomes were found in the tumor cells: Reassociation kinetics with tumor cell DNA using *in vitro*  $^3\text{H}$ -labelled EBV-DNA should have allowed the detection of one EBV genome per ten cells [8]. In addition, *in situ* hybridization with frozen sections of the biopsy was performed using  $^3\text{H}$ -labelled EBV-DNA as in the reassociation kinetics, a procedure similar to that employed for detection of EBV-DNA in nasopharyngeal carcinoma [9]. This method should have allowed the detection of a few cells carrying EBV genomes scattered in the tissue, even though the average number of EBV genomes per total biopsy cells would not have permitted their detection by reassociation kinetics. EBNA, another important viral marker, could not be demonstrated within the tumor cells by immunofluorescence technique [10] examining more than 1000 cells.

However, on morphological examination, the diagnosis of a lymphogranulomatosis from the epitheloid cell type was established. According to the Kiel classification, this is a rare entity with poor prognosis and an abundance of epitheloid and Reed-Sternberg cells [11]. Staging procedures including exploratory laparotomy and splenectomy revealed a stage I A according to the Ann Arber criteria. Therefore, radiotherapy was initiated.

## DISCUSSION

This case is remarkable for several reasons. HD developed during the late convalescent phase of IM as documented by serial histological examinations of involved lymph nodes and rising and falling EBV antibody titers. Although from a clinical point of view the correlation between IM and subsequent HD seems to be quite obvious, it does not necessarily imply that EB virus is oncogenic. As in the African Burkitt's lymphoma or in the nasopharyngeal carcinoma, a prerequisite for this possibility would be the presence of viral footprints within the tumor cells. Despite an extensive search in a large tumor mass

using very sensitive methods, neither EBV-DNA nor EBNA could be detected.

On the other hand, the typical pattern of IM heterophil and EBV antibody establish beyond doubt the diagnosis of preceding IM in our patient, whose clinical symptoms might have been uncharacteristic at the time of presentation. The most specific and sensitive marker for IM is the detection of antibodies against the EBV-VCA. The specific IgM antibodies are valuable early diagnostic tools, since their presence denotes in all probability a current primary infection [12]. VCA-IgG antibodies appear within five to seven days of infection and remain positive for life [13]. The measurement of this antibody is usually of no help in determining the diagnosis of initial infection unless one is able—as in our case—to catch that fleeting point of a rise in serological titer. EBV-EA antibodies have proven to be of considerable diagnostic value, since appearance of this antibody is transitory in IM and practically never seen in healthy donors [12]. Taking the complete serological pattern of our patient into account (Fig. 1)

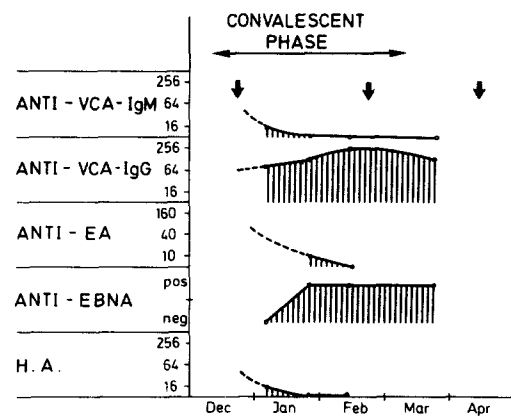


Fig. 1. Schematic presentation of antibody responses to Epstein-Barr virus. The normal values in our laboratory for a normal population are: anti-VCA-IgM, 1:4; anti-VCA-IgG, 1:64; anti-EA, 1:8; H.A., 1:4. Values above these titers are considered positive; however, the complete pattern of antibody titers has to be taken into account for the diagnosis of IM. H.A. = heterophil antibody. The arrows indicate the serial histological examinations.

together with a history of a "common cold" during the month of October, one could pinpoint the stage of IM at first presentation as being in the late convalescent phase.

An association between IM and the development of certain lymphoproliferative diseases has been postulated [14] and supported by several seroepidemiological studies [1, 13]. Limitations of most of these studies are that they are too small to be interpreted with

confidence and that the diagnosis of IM was not well documented. One study in which both limitations were overcome [2] shows a four-fold increase in rate of HD among people who had IM. The excess risk was greatest within three years of diagnosis of IM. A possible explanation for their association is that IM and HD might have a common cause such as an immune deficiency state. Another alternative is that IM may promote the rapid development of HD in a person in whom the

disease is latent or progressing slowly. The point of this report is to show using sensitive microbiological tests that there seems to be no direct oncogenic relationship between EBV and HD, because no EB viral marker could be identified in the tumor cells. This situation contrasts markedly to the one described for Burkitt's lymphoma and nasopharyngeal carcinoma, although in either case, EB virus might well be an important risk factor in the development of malignant diseases.

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