Commentary

EB Virus, Infectious Mononucleosis, and Cancer: The Closing of the Web

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The web of causation is closing in on EB virus in its relation to certain conditions. In heterophile positive infectious mononucleosis the web of causation seems established beyond any reasonable doubt (1–3). The evidence derived from prospective serological studies that EBV antibody is regularly absent prior to infectious mononucleosis and regularly appears during illness (4–7) is supported by the demonstration of EBV-specific IgM antibody indicative of a primary infection (8–10), by identification of the virus in lymphocytes cultured from the blood (1) by isolation of the virus from the throat (11–13), and by suggestive experimental evidence of infectious mononucleosis in squirrel monkeys (14) and gibbons (15) inoculated with EBV or EBV-infected cells. The immunological events that turn infectious mononucleosis on and turn it off are not so clear, although evidence is rapidly accumulating on these questions. Figure 1 presents a working schema of pathogenesis.

EB virus appears to enter the oropharynx in young adults, probably thru kissing, and multiplies locally producing a lytic and persistent infection (16). It enters the blood stream and possibly the gut although the latter has not yet been established. Lymphocytes of the B-type are infected and a nonproductive infection of long duration is established. Some of these transformed B cells may contribute early to the atypical lymphocytosis. Current evidence then suggests that there is mixed lymphocyte reponse of T cells to B cells altered by an EBV-membrane induced antigen. This results in T cell proliferation (17), transformation, and a major outpouring of atypical lymphocytes. Lymphocytes from acute cases of infectious mononucleosis have also been found to cause stimulation of convalescent leukocytes from the same

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persons indicating the presence of a new surface antigen during acute illness (18–19). While it seems most probable that the immunologic response of the T cells is the major cause of the atypical lymphocytes of infectious mononucleosis (17–20)—this needs additional proof. The activated T cells may cause the destruction of the EBV-infected B cells, as has been shown in vitro and in other viral systems (21, 22). This destruction may account for the inability to demonstrate EBV or its genome in fresh lymphocytes from acute cases of 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 (16). The mechanism of heterophile production is still unexplained but the knowledge that its appearance is most common in EBV infections of young adults (2) and that the age of the donor of the lymphocytes is important in the degree of expression and release of EBV suggest avenues of investigation using new techniques capable of identifying in vitro antibody and antigen production by lymphocytes from different ages. For example, EBV-infected fetal lymphocytes do not have demonstrable VCA antigen but do contain EB virus nuclear antigen (EBNA) (23) and complement fixing antigen (13). The EB nuclear antigen is demonstrated by the fixation of complement in an indirect immunofluorescent test (23). In lymphocytes from young adults and from marmosets EBV may mature more fully releasing antigens to other lymphocytes. Heterophile antibody may be produced in response to membrane-in-

2 Very recently Zur Hausen et al. have demonstrated EBV genome in the peripheral lymphocytes of 2 severe case of infectious mononucleosis (Zur Hausen, H., Schulte-Holthausen, H., Wolf, H., Dörries, K., and Egger, H., Attempts to detect virus-specific DNA in human tumors. II. Nucleic acid hybridizations with complementary RNA of human herpes group viruses, Int. J. Cancer 13, 657–664, 1974).
duced changes of EBV which may express themselves more fully in lymphocytes of young adults than in younger children or in fetal lymphocytes. There is no experimental data yet in support of this concept.

The causal relationship of EB virus to Burkitt’s lymphoma is more indirect than in infectious mononucleosis because of the difficulty in mounting a meaningful prospective study in which temporal relationships between EBV and the tumor can be recognized. However, a pilot investigation of this type has been launched in the West Nile area of Africa involving 1122 children who are being serially followed and bled (24).3 A large number of persons are needed because of the relatively low incidence of Burkitt’s lymphoma, averaging about 13 per 100,000 in children ages 4–8 in this area. Furthermore, the prolonged follow-up required to identify cases of Burkitt lymphoma make this a major undertaking. The evidence currently supporting a causal role of EB virus in Burkitt lymphoma and its shortcomings, has been summarized in an excellent review by Miller (25) and synthesized into a working hypothesis by Epstein and Achong (16). In African Burkitt lymphoma, the major facts suggesting EB virus as the cause are: (1) the presence of EBV antibody in the sera of almost all cases; (2) the demonstration of very high antibody titers in the sera of 80% or more of the cases as compared to healthy controls and most other lymphoproliferative diseases; (3) the breadth of the antibody response including antibodies to viral capsid, complement fixing, membrane, and early antigens as well as neutralizing antibody; (4) the demonstration of the EBV genome in freshly obtained biopsy specimens of tumor tissue by nucleic acid hybridization studies (26, 27) and/or of EB nuclear antigen (23) from a total of about 120 cases; (5) the experimental production of reticulum cell sarcomas in cotton-top marmosets using EBV-infected marmoset cells or cell-free EBV by Shope et al. (28) with the ability to recover EBV from these tumor cells; and similar results in owl monkeys (29); (6) the capacity of EB virus to transform normal lymphocytes into cells with an infinite potential for proliferation in vitro. This last property has been termed “immortalizing” factor by Miller (25) who presents a detailed study of this event in this issue of The Yale Journal of Biology and Medicine (p. 123). Here the question of the expression of EB virus in 3 lymphoblastoid lines is examined. The results suggest that the capsid antigens are discontinuous in expression in contrast to the complement fixing and nuclear antigens which are continuously expressed. An understanding of the behavior of EBV in productive and nonproductive cell lines may provide clues to their in vivo counterparts.

The web of causation is thus tightening on the causal relation of EB virus to Burkitt lymphoma. It comes close to fulfilling the Henle–Koch postulates. There is also increasing evidence of the oncogenicity of herpes viruses in several animal species: of lymphoma in New World monkeys inoculated with herpes saimiri or ates viruses, of Marek’s disease, a herpes-associated lymphoproliferative disease in chickens, of Lucké adeno carcinoma in frogs, and of herpes sylvilacus in cotton-tail rabbits which produces lesions resembling a malignant lymphoma.4 But there

3 Thirty-five thousand children are now under study and Burkitt lymphoma has occurred in 5 thus far (R. Morrow, personal communication).

4 See “Oncogenesis and Herpesviruses,” (P. M. Biggs, G. deThé and L. N. Payne Eds.), IARC (Lyon 1972) and “The Herpesviruses,” (A. Kaplan Ed.), Academic Press, New York and London, (1973) for information on herpes viruses of man and animals and their oncogenic potential.
are problems in fully accepting EBV as the sole cause of Burkitt lymphoma. First, evidence suggesting the presence of a RNA tumor virus in Burkitt lymphoma cells by Kufe et al. (30) raises the question as to whether this virus and EBV may be working in concert, or one activating the other, or one or both being only passenger viruses. Second, biopsies from a few cases of African Burkitt lymphoma have lacked detectable EBV genome (23–27) and antibody has been absent in the sera of 2 cases. Furthermore, in American patients diagnosed histologically as Burkitt lymphoma EBV antibody has been absent from the serum of 19% of the cases in 2 separate studies (31–32), present but without elevation in titer in many others (31–33), and without demonstrable EBV genome in the biopsy tissues of 4 American Burkitt lymphoma cases (34). In addition to EB virus as a causative agent, there is strong epidemiologic evidence incriminating malaria as an important cofactor in African Burkitt lymphoma to account for the high incidence of Burkitt lymphomas in Africa and New Guinea (35, 36). The mechanism for this enhanced susceptibility is not clear but might be due to antigen-antibody malarial complexes (blocking factors) interfering with cell-mediated (T lymphocyte) responses to EBV infection resulting in viral persistence and tumor formation. The study of cell-mediated immunity in Burkitt lymphoma is just beginning. Fass et al. (37) have shown delayed hypersensitivity reactions in Burkitt lymphoma patients exposed to autologous tumor cell extracts, but it is not known whether these reactions are directed against EBV-induced antigens or not. The positive reactions correlated with sustained remissions.

The web of causation for EBV in nasopharyngeal carcinoma is of the same general order as in African Burkitt lymphoma: EBV antibody is almost universally present in the serum, the antibody titer is very high in 80% of the cases and the EBV genome has been demonstrated not only in the lymphocyte infiltrated tumor (26) but in the epithelial cells themselves (38). A lead as to how EB virus produces infection in epithelial cell in vivo when it is usually limited to lymphocytes in vitro has been provided by the demonstration that hybrid cells consisting of an epithelial line and BL lymphocytes fused with Sendai virus, and stimulated by BUDR, support the persistence of EBV genome (39). The possible existence of an in vivo fusion factor has been suggested by the occurrence of elevated antibody titers to herpes simplex type 1 (40a) and to parainfluenza type 3 viruses (40b) in sera from cases of nasopharyngeal cancer.

EB virus and a cofactor virus producing fusion are 2 potential ingredients in the causation of nasopharyngeal cancer. A third important ingredient is genetic susceptibility. It was early recognized that persons of Chinese descent, especially from southern China, were at 10–50 times the risk of developing nasopharyngeal cancer than caucasians in the same environment (41, 42). This high susceptibility held true even when such Chinese lived in Australia (43), New South Wales (44), or Hawaii (45). Important support for this genetic role has recently been derived by Simons et al. (46) in 2 carefully controlled analyses of human leukocyte antigen types in nasopharyngeal carcinoma. They found there was a significant increase in the proportion of Chinese patients with nasopharyngeal cancer (NPC) in whom less than 2 second locus antigens were detectable as compared to Chinese without NPC. In addition they found a significant elevation in the frequency of HL-A2 antigen in Chinese patients with NPC as compared to Chinese persons without NPC.

A higher frequency of raised antibody titers to EB virus and a higher geometric mean antibody titer have also been found in patients with Hodgkin’s disease
(47–51), systemic lupus erythematosus (52), and sarcoidosis (53, 54) than in comparable healthy controls. Some investigators, however, have failed to find elevated EBV titers in SLE (55, 56); the differences may be of a technical nature or related to the activity of the disease at the time the sample was taken (57). In systemic lupus erythematosus, significant elevations in rubella, parainfluenza, and measles viral antibody titers as compared to matched controls have also been found (52, 58–60), and the first two antibodies have also been raised over matched controls in patients with sarcoidosis (54). In contrast to this, antibody titers to influenza A and B, cytomegalovirus, respiratory syncytial, herpes simplex, adenoviruses, and other viruses have not been raised in these two conditions. It should be emphasized that the frequency, height, and breadth of EBV antibody response in Hodgkin’s disease, SLE, and sarcoidosis have been less than in Burkitt lymphoma (BL) or nasopharyngeal carcinoma (NPC). For example, only 30–40% of patients with the 3 former conditions have elevated EBV antibody titers as compared to 60–80% in NPC and BL and the geometric mean titer in those with antibody is only \( \frac{1}{4} \) to \( \frac{1}{2} \) of that found in the 2 malignancies. There is some evidence that HLA differences may play a role in Hodgkin’s disease (50, 61, 62) and in systemic lupus erythematosus (63), and that a serum inhibitor of cell-mediated immunity may be present in sarcoidosis (64). The genome of EBV was not detected in the biopsy tissues of 4 American patients with Hodgkin’s disease (34, 65). More search for the genome of EBV and for EBV nuclear antigen must be made in tissues of Hodgkin’s disease and other conditions with high EBV titers before any conclusion can be drawn. At present, the relationship of EB and other viruses to the pathogenesis of Hodgkin’s disease, SLE, and sarcoidosis is much weaker than that of EBV to Burkitt lymphoma and nasopharyngeal cancer. The raised antibody titers may be secondary to depressed cell-mediated immunity and the viruses may play no role in causation. Skepticism has recently been expressed on the causative role of EBV and Hodgkin’s disease (50) especially as antibody elevations occur only in certain types, mainly in lymphocyte depletion and mixed-cellularity types. However, in diseases of unknown causation, it is important to explore every lead carefully before making premature decisions to exclude a possible causative agent.

The presence of high EBV antibody titers in all these diseases suggests an antigenic stimulation by viral persistence and/or reactivation. The absence of elevated antibody titers under certain conditions is compatible either with the absence of viral activity or with an excess of viral antigen over antibody, such that immune complexes form which reduce the measurable antibody level. This phenomenon has been suggested to account for the lower EBV antibody titers during the active phases of SLE (52) and in immune complex nephritis (66). Low or absent circulating antibody has also been observed in mice in lymphocytic choriomeningitis, in murine leukemia, and in lactic acid dehydrogenase-virus immune complex glomerulonephritis (67–70).

A selected impairment in cell-mediated immunity has been proposed as a common denominator in EBV-associated infections (71, 72) resulting in virus persistence or reactivation. The mechanism by which this might occur is not entirely clear but increasing attention is being directed to the T lymphocyte in its role in the recognition and control of viral infections and of their reactivation. The T lymphocytes may be deficient in numbers, tied up in other tasks (antigenic competition), genetically lack appropriate receptors for EBV or EBV-induced antigens, be blocked in their task by serum inhibitors, or rendered ineffective by immuno-
suppressant drugs. Suppressor T cells may block the action of other T cells. Intensive study of cell-mediated immunity in EBV infections is in order with special focus on whether T cells respond normally to EB virus in infectious mononucleosis, Burkitt lymphoma and nasopharyngeal cancer as well as in other EBV-associated conditions. A method for such stimulation has been published by Gerber and Lucas (73). It has been recently shown that patients who are immunosuppressed during renal transplantation or for therapeutic reasons excrete EB virus from the pharynx more commonly than controls, and that the infection may be reactivated (74). There is clearly an increased risk for certain types of cancer in persons given immunosuppressant drugs in high dosage (75). This is of the order of 35 times higher than normal, particularly for reticulum-cell sarcoma.

The presence of high antibody levels to certain viruses thus appears to be associated with an increased risk to some malignancies, to chronic diseases like SLE and sarcoidosis, as well as to chronic neurologic diseases like progressive multifocal leukoencephalopathy (papovavirus), subacute sclerosing panencephalitis and multiple sclerosis (both associated with measles virus). The critical evidence lacking is whether these high titers precede the disease and thus play a causal role, accompany the disease expressing a common immunologic defect with the real cause of the disease, or follow in the wake of the disease as a consequence of depressed cell-mediated immunity induced by the disease itself. This temporal sequence may vary in different diseases. The retrospective approach also fails to identify other possible diseases associated with high antibody titers and does not permit assessment of risk factors. It is important now to mount large scale prospective seroepidemiologic studies which would (1) identify healthy individuals who have high antibody titers to EBV, rubella, parainfluenza, measles, papova virus, herpes type 2 viruses etc. and/or have impaired lymphocyte responses to these viruses; (2) seek evidence of viral excretion and persistence; (3) follow such persons and matched controls, who have normal antibody levels, for the occurrence of cancer, chronic diseases, and neurological disorders, especially those of unknown cause associated with defects in cell-mediated immunity or in which immune complexes are suspected as playing a role in the pathogenesis; (4) carry out intensive virological and immunological studies when a chronic disease occurs, including a search for the virus or viral genome in affected tissues. The results should be compared with matched controls, whenever possible. If one takes the total incidence of all of the malignant and chronic diseases in which viruses may play a role, then the return from such a costly venture should make it justifiable and make the population base manageable. The populations served by large health plans caring for all ages would be an admirable start because of their accessibility to bleeding on entry into the plan and at periodic intervals thereafter and because of the possibility of good clinical surveillance. Indeed, it might not be necessary to carry out viral antibody tests until a disease develops provided frozen serum and lymphocyte samples were available prior to this time from the patient and from suitable controls.

From the epidemiologist's standpoint there are factors that might obscure a true causal relationship between EB virus and the chronic conditions with which it is

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1 Prospective analysis of cancer and chronic diseases according to their HLA type should also be included, if possible.

2 The major ones of current interest are lymphomas, leukemia, cervical and penile cancer, SLE, rheumatoid arthritis, sarcoidosis, immune complex, kidney disease, chronic neurological diseases.
associated. EBV might be a causal factor in one setting but not in another. For example, it might cause African Burkitt lymphoma but not American Burkitt lymphoma; it might be a cause of Hodgkin's disease in the young age group but not in the old; it might be causally related to some cases of systemic lupus erythematosus but not to others. There may be several strains of EBV some of which are oncogenic and some of which are not. As a candidate oncogenic virus for 2 special malignancies, Burkitt lymphoma in Africa and nasopharyngeal carcinoma in Chinese, EB virus appears to have won the primary (76). Full acceptance will require much more evidence to satisfy everyone. Virologically, this includes further attempts to produce lymphomas in primates using purified EBV derived from a Burkitt lymphoma and in the presence or absence of concomitant experimental malaria; assessment of the role, if any, of an RNA virus in this setting, and expanded efforts to identify the EBV genome and/or nuclear antigen in various tissues from patients with lymphoma, NPC, other cancers, and controls. Epidemiologically, prospective surveys must establish that EBV infection and high antibody titers precede the disease and that such high titers are associated with a higher risk of tumor than in those with normal antibody titers; control of a cofactor such as malaria in Burkitt lymphoma or a myxovirus infection in NPC should decrease the incidence of the associated tumor. Genetically, the relation of HLA and similar antigens to NPC must be clarified and the role of these genetic attributes explored in other conditions associated with high EBV antibody levels.

Immunologically, the mechanism of viral persistence and/or reactivation in these conditions need further analysis, particularly the specific responses of lymphocytes to EBV, myxoviruses, and malaria. Therapeutically, the possible effect of transfer factor derived from lymphocytes of EBV-immune subjects should be cautiously explored in controlled trials of Burkitt lymphoma and nasopharyngeal carcinoma as compared with transfer factor from the lymphocytes of persons lacking EBV antibody.

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