The Past Is Prologue: Use of Serum Banks in Cancer Research

Alfred S. Evans and Nancy E. Mueller

Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut 06510 [A. S. E.], and Department of Epidemiology, Harvard University School of Public Health, Boston, Massachusetts 02115 [N. E. M.]

Abstract

In this paper, we emphasize the uses of serum banks in cancer research. These include not only case/control studies but also prospective seroepidemiological studies in which the development of a serological marker, such as a viral antibody or viral antigen, can be correlated with the subsequent development of cancer in either an active surveillance program or the use of cancer registries or hospital records. Several different methods of application of the cohort technique are illustrated by studies of hepatitis B antigen and hepatocellular carcinoma and of Epstein-Barr virus in relation to African Burkitt's lymphoma, Hodgkin's lymphoma, and non-Hodgkin's lymphoma. Collections of sera done for one purpose can often be utilized for another purpose, if properly stored and documented. Two examples are tests for human T-cell leukemia virus, type 1, antibody from sera done for a health survey in Barbados approximately 8 years earlier and the use of data determined for a prospective study of the incidence of Epstein-Barr virus infection and infectious mononucleosis in West Point Cadets for psychological factors affecting the development of clinical illness among those infected. Archival materials, such as frozen tissues and paraffin sections, may also now be utilized for identifying genomes of potential oncogenic viruses by the polymerase chain reaction.

Introduction

This discussion will focus on the uses of stored serum samples and other stored materials in epidemiological research, with special reference to their application to cancer research. The applicability of stored serum samples to cancer epidemiology depends on the availability of some laboratory marker in serum or plasma that is measurable and which will survive the frozen state. For viruses potentially involved in cancer pathogenesis, these markers include antibody and antigens, such as HBV. An important new development is the ability of the PCR to detect such antigens in material that has been frozen. This includes not only sera and plasma but also lymphocytes, frozen tissue sections, and paraffin blocks. In chronic diseases, stored serum samples are also suitable for markers such as cholesterol, lipids, hormones, and various chemical indicators of the nutritional state. Several uses of archival material will be discussed.

Case/Control and Prospective Serological Studies

While case-control studies of the prevalence of antibodies or antigens present in stored serum samples have provided useful clues to the possible relationship of a virus to a tumor, they do not reveal whether the viral marker was the result of the cancer or preceded it as a risk factor in its development. For this reason, we wish to place primary emphasis on prospective seroepidemiological studies and the methodology of such studies.

2 To whom requests for reprints should be addressed, at the Department of Epidemiology, Harvard University School of Public Health, 677 Huntington Avenue, Boston, MA 02115.
3 The abbreviations used are: HBV, hepatitis B virus; PCR, polymerase chain reaction; HCC, hepatocellular cancer; HBsAg, hepatitis B surface antigen; EBV, Epstein-Barr virus; NHL, non-Hodgkin's lymphoma; HTLV, human T-cell leukemia virus, type 1.
that of controls had a 30-fold greater risk of developing Burkitt’s lymphoma. No risk was related to other EBV antibodies or to antibodies to cytomegalovirus or measles virus.

A variation of this method of a planned prospective cohort study with a case/control analysis is one in which the development of the tumor is ascertained from cancer registries or hospital records rather than through an active surveillance system. This technique was applied to the possible relationship between EBV and Hodgkin’s disease. While case/control studies, such as those the authors were involved in Boston, MA (3), and in Sao Paulo, Brazil (4), had clearly established that approximately 30–40% of patients with Hodgkin’s disease had significantly higher EBV-IgG antibody titers than did age- and sex-matched controls, it was not known whether these altered patterns were the consequence of the disease itself or whether they preceded the development of the tumor and might play a causal role in its pathogenesis. Because of the rarity of the tumor, a large cohort was needed to explore this temporal pattern. The persons were followed up through the Washington County Cancer Registry and 2 cases of Hodgkin’s disease showed significant, but different, altered patterns of EBV antibody as compared with controls, suggesting a possible causal role of EBV in some of the lymphomas but different pathogenetic mechanisms. These serological findings have been strengthened by the PCR finding of an EBV genome in Reed-Sternberg cells from about half the cases of Hodgkin’s disease.

between serum collection and the development of the tumor was 4 years. The sera were matched with 1 or 2 age- and sex-matched controls bled at the same time who did not develop cancer. The samples were coded and arranged in random sets at Harvard University and sent to Yale University where they were tested in the laboratory of the WHO Serum Bank as unknowns for various antibodies to EBV. The immunofluorescent tests were read independently by one of us (A. S. E.) and a technical associate; cytomegalovirus antibodies were also determined by an enzyme-linked immunosorbent assay test. The results were forwarded to Harvard University for computer analysis, and the code was not broken until the end of the study. Because this assigned topic at this conference was to discuss the use of archival materials, and not the results of such studies, which will be presented in another paper, (6–8), the results will not be given here in detail. In brief, sera obtained prior to the development of Hodgkin’s disease and non-Hodgkin’s lymphoma showed significant, but different, altered patterns of EBV antibody as compared with controls, suggesting a possible causal role of EBV in some of the lymphomas but different pathogenetic mechanisms. These serological findings have been strengthened by the PCR finding of an EBV genome in Reed-Sternberg cells from about half the cases of Hodgkin’s disease.

between serum collection and the development of the tumor was 4 years. The sera were matched with 1 or 2 age- and sex-matched controls bled at the same time who did not develop cancer. The samples were coded and arranged in random sets at Harvard University and sent to Yale University where they were tested in the laboratory of the WHO Serum Bank as unknowns for various antibodies to EBV. The immunofluorescent tests were read independently by one of us (A. S. E.) and a technical associate; cytomegalovirus antibodies were also determined by an enzyme-linked immunosorbent assay test. The results were forwarded to Harvard University for computer analysis, and the code was not broken until the end of the study. Because this assigned topic at this conference was to discuss the use of archival materials, and not the results of such studies, which will be presented in another paper, (6–8), the results will not be given here in detail. In brief, sera obtained prior to the development of Hodgkin’s disease and non-Hodgkin’s lymphoma showed significant, but different, altered patterns of EBV antibody as compared with controls, suggesting a possible causal role of EBV in some of the lymphomas but different pathogenetic mechanisms. These serological findings have been strengthened by the PCR finding of an EBV genome in Reed-Sternberg cells from about half the cases of Hodgkin’s disease.

that of controls had a 30-fold greater risk of developing Burkitt’s lymphoma. No risk was related to other EBV antibodies or to antibodies to cytomegalovirus or measles virus.

A variation of this method of a planned prospective cohort study with a case/control analysis is one in which the development of the tumor is ascertained from cancer registries or hospital records rather than through an active surveillance system. This technique was applied to the possible relationship between EBV and Hodgkin’s disease. While case/control studies, such as those the authors were involved in Boston, MA (3), and in Sao Paulo, Brazil (4), had clearly established that approximately 30–40% of patients with Hodgkin’s disease had significantly higher EBV-IgG antibody titers than did age- and sex-matched controls, it was not known whether these altered patterns were the consequence of the disease itself or whether they preceded the development of the tumor and might play a causal role in its pathogenesis. Because of the rarity of the tumor, a large cohort was needed to explore this temporal relationship in a prospective study. We thought it might be possible to utilize serum collections already in existence and in which a follow-up for tumor development was possible through a cancer registry or hospital records. In a pilot study which one of us (A. S. E.) carried out with G. Comstock (5), sera from about 26,000 adults were collected for possible clues to cancer. The persons were followed up through the Washington County Cancer Registry and 2 cases of Hodgkin’s disease were identified. Sera from these 2 persons were tested in a coded fashion for EBV antibodies along with age- and sex-matched controls whose blood had been sampled at the same time. The results showed significant elevations in EBV antibody titers in Hodgkin’s cases as compared with the controls. Encouraged by these preliminary results, we embarked on a much larger study based on 4 serum banks in 3 countries involving >241,500 serum samples and with a broadened objective to seek the possible relationship of EBV not only to Hodgkin’s disease but also to non-Hodgkin’s lymphoma, nasopharyngeal carcinoma, leukemia, and multiple myeloma. Table 4 presents data concerning the sources of the collections and the number of the targeted tumors found. The average time

between serum collection and the development of the tumor was 4 years. The sera were matched with 1 or 2 age- and sex-matched controls bled at the same time who did not develop cancer. The samples were coded and arranged in random sets at Harvard University and sent to Yale University where they were tested in the laboratory of the WHO Serum Bank as unknowns for various antibodies to EBV. The immunofluorescent tests were read independently by one of us (A. S. E.) and a technical associate; cytomegalovirus antibodies were also determined by an enzyme-linked immunosorbent assay test. The results were forwarded to Harvard University for computer analysis, and the code was not broken until the end of the study. Because this assigned topic at this conference was to discuss the use of archival materials, and not the results of such studies, which will be presented in another paper, (6–8), the results will not be given here in detail. In brief, sera obtained prior to the development of Hodgkin’s disease and non-Hodgkin’s lymphoma showed significant, but different, altered patterns of EBV antibody as compared with controls, suggesting a possible causal role of EBV in some of the lymphomas but different pathogenetic mechanisms. These serological findings have been strengthened by the PCR finding of an EBV genome in Reed-Sternberg cells from about half the cases of Hodgkin’s disease.

that of controls had a 30-fold greater risk of developing Burkitt’s lymphoma. No risk was related to other EBV antibodies or to antibodies to cytomegalovirus or measles virus.

A variation of this method of a planned prospective cohort study with a case/control analysis is one in which the development of the tumor is ascertained from cancer registries or hospital records rather than through an active surveillance system. This technique was applied to the possible relationship between EBV and Hodgkin’s disease. While case/control studies, such as those the authors were involved in Boston, MA (3), and in Sao Paulo, Brazil (4), had clearly established that approximately 30–40% of patients with Hodgkin’s disease had significantly higher EBV-IgG antibody titers than did age- and sex-matched controls, it was not known whether these altered patterns were the consequence of the disease itself or whether they preceded the development of the tumor and might play a causal role in its pathogenesis. Because of the rarity of the tumor, a large cohort was needed to explore this temporal relationship in a prospective study. We thought it might be possible to utilize serum collections already in existence and in which a follow-up for tumor development was possible through a cancer registry or hospital records. In a pilot study which one of us (A. S. E.) carried out with G. Comstock (5), sera from about 26,000 adults were collected for possible clues to cancer. The persons were followed up through the Washington County Cancer Registry and 2 cases of Hodgkin’s disease were identified. Sera from these 2 persons were tested in a coded fashion for EBV antibodies along with age- and sex-matched controls whose blood had been sampled at the same time. The results showed significant elevations in EBV antibody titers in Hodgkin’s cases as compared with the controls. Encouraged by these preliminary results, we embarked on a much larger study based on 4 serum banks in 3 countries involving >241,500 serum samples and with a broadened objective to seek the possible relationship of EBV not only to Hodgkin’s disease but also to non-Hodgkin’s lymphoma, nasopharyngeal carcinoma, leukemia, and multiple myeloma. Table 4 presents data concerning the sources of the collections and the number of the targeted tumors found. The average time
and in tumor tissue from almost 20% of those with non-Hodgkin's lymphoma (9–12). These observations have, in part, been dependent on the applicability of the PCR technique to archival materials such as frozen or paraffin sections of lymphoma tissues. This use of PCR will be discussed in detail in other papers in this symposium. Our serological results from a number of pretumor serum samples from cases of nasopharyngeal cancer (NPC) failed to reveal EBV-IgA antibody prior to diagnosis (8), in contrast to suggested evidence from screening studies in China, in which it was present in some cases prior to the clinical diagnosis of the tumor (13).

**Multipurpose Uses of Archival Material**

The WHO has emphasized the usefulness of carrying out multiple tests on materials from a single seroepidemiological survey (14). We have carried out many such surveys to define the patterns of infectious diseases and the need for vaccination in many South American and Caribbean countries, the most recent of which done in Barbados (15) and St. Lucia (16).

It is also possible to carry out studies on stored serum samples for purposes other than those originally planned. Two examples of this are: (a) the serum collections made in Barbados (15) in 1972 from about 1000 persons of all ages in 250 households were retested in 1980 for HTLV-1 antibody in collaboration with the Viral Epidemiology Section of the National Cancer Institute (17). This became possible after HTLV-1 was discovered in 1981 (18) and tests for antibody became available. Our results indicated an antibody prevalence of about 4.5% for HTLV-1 in the Barbados population sampled, approximately 8 years prior to the discovery of the virus. The rate increased with age and was 14% in persons whose sera were positive for VDRL antibody versus 3.5% in those whose sera were VDRL negative, which is indicative of sexual transmission of the virus. We are currently engaged in clinical and serological follow-up of those infected approximately 17 years ago and of their family members.

(b) is based on a prospective study of the relationship of EBV to infectious mononucleosis carried out on the entire entering cohort of approximately 1400 cadets at the West Point Military Academy during a 4-year period (19). During that period almost half of the susceptible cadets (those lacking EBV antibody) became infected, as demonstrated by the appearance of EBV antibody, but only approximately 25% of these developed clinical infectious mononucleosis. We were interested in why some did and some did not develop clinical illness. We learned later that a psychological profile had been carried out on the same cohort as we were studying as part of a countrywide analysis of psychosocial characteristics of students entering college. Using our EBV data set, and with the help of Stan Kasl, a behavioral epidemiologist at Yale University, we sought correlations between the behavioral characteristics recorded in the questionnaire and our data concerning the incidence of clinical infectious mononucleosis among those infected with EBV (20). Such determinants have been called the "clinical illness promotion factors" (21). As shown in Table 3, commitment to a military career was one. If the cadet was highly motivated, and particularly if he were the son of an overachieving father, the incidence of clinical infectious mononucleosis following infection was much higher (57.1%) than in those cadets with low motivation (10.7%). Similarly, those with high motivation who were doing poorly academically in the third or fourth year, as compared with the previous year, had a 50% incidence of infectious mononucleosis among those infected versus only 5.6% in those who were doing better than in the previous year. The picture which emerged of those at highest risk of clinical disease after infection was that of the highly committed son of an overachieving father who was working hard but failing academically. These same stress factors also affected the duration of illness, as well as the frequency of heterophile antibody in those with subclinical infection, an objective laboratory finding that could not be influenced by the attitude of the cadet toward reporting or not reporting illness. These effects of stress operating on the response of the host to primary infection with EBV have also been shown to be operative in influencing the reactivation of EBV by the Glavers (22–24). They found in casecontrol studies that significantly higher geometric mean EBV antibody levels were present in 38 recently separated or divorced couples than in 38 age- and sex-matched married couples (463 versus 149) (22); very high antibody levels were also demonstrated in caretakers of patients with Alzheimer's disease versus matched controls (641 versus 376) (23). Of special interest for the older age group at risk to non-Hodgkin's disease was the observation that the geometric mean EBV IgA antibody titer was 662 in 44 geriatric patients (average age, 77 years) as compared with 94 in 70 medical students (average age, 23 years) (24).

One of us (A. S. E.) suggested that the effects of stress and depression on immunoregulation may lead to EBV reactivation and that the high antibody titers seen in most cases of the chronic fatigue syndrome are the result of this reactivation rather than the reverse, in which EBV was the cause of the syndrome (25). It seems quite possible that stress and/or depression could play a role in the depressed immune function that accompanies non-Hodgkin's lymphoma, particularly the "spontaneous" cases not known to be associated with the drug-induced immunosuppression in transplant patients or in persons with human immunodeficiency virus infection. The pattern of EBV antibodies that we have recently demonstrated in sera obtained several years prior to the development of spontaneous non-Hodgkin's lymphoma (6) is similar to that found when NHL develops and is present in other immunodepressed states. This early immunosuppressive event, which precedes the appearance of NHL, may be an initiating factor in its development and may well be the consequence of some stressful or depressive experience. It is not possible to determine whether this is true or not from our data base, but we urge future epidemiological studies of spontaneous NHL to include questions that would reflect such life events in the questionnaire. They were included in the two fine casecontrol studies presented at this meeting, which revealed few clues to the pathogenesis of NHL, except the possible influence of exposure to environmental toxins. Further studies of the effects of stress and depression on immune function and on viral reactivation in the age groups at highest risk for NHL would be desirable. In this older population, such stressful life events as death of a spouse, loss of job, sale of a house and disposal of cherished possessions, movement into a retirement or nursing home, and care for a seriously ill or disabled spouse are all events that deserve study by the epidemiologist, virologist, immunologist, and the psychologist working together to seek clues to the causes of NHL.

Finally, we wish to encourage the collection and storage of serum, lymphocytes, and tissues in both casecontrol and prospective studies of viruses and cancer, as well as sera from healthy high-risk populations in a health systems so that the subsequent occurrence of cancer and other chronic conditions can be identified through disease registries or computerized
USE OF SERUM BANKS

hospital and clinic records. In the past, the WHO established serum reference banks primarily directed at preserving archival material derived from surveys for infectious diseases. These were located in Prague, Czechoslovakia, New Haven, CT., and Tokyo, Japan, to serve the various regions of the world. The one at Yale University contained sera from approximately 50,000 persons derived from health and serological surveys in the Americas. These included military recruits, Peace Corps volunteers, and civilians in the United States and several South American and Caribbean countries. This entire collection has now been transferred to the Viral Epidemiology Section of the National Cancer Institute where it will ultimately be available for their use, as well as that of other investigators interested in archival materials for research into cancer or any other disease.

In summary, the emergence of human immunodeficiency virus as a worldwide problem and the increasing recognition of the importance of certain viruses in the pathogenesis of some cancers have both contributed to the rebirth of seroepidemiological techniques and of the use of archival materials in epidemiological practice and research.

References


The Past Is Prologue: Use of Serum Banks in Cancer Research

Alfred S. Evans and Nancy E. Mueller


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/52/19_Supplement/5557s

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.