Approaches to Immunological Control of Virus-associated Tumors in Man: Introductory Remarks

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The rapidly accumulating evidence for the role of viruses in the etiology of human cancer makes the topic of antiviral agents, such as vaccines, of great interest to tumor immunologists. Since direct proof of oncogenicity in humans is impossible, however, a discussion of the means of controlling human tumors that are potentially virus induced must be speculative and draw primarily on observations taken from apparently relevant animal models. Three groups of viruses, known to cause tumors in animals, have also been suspected of causing cancer in man: the DNA herpesviruses, the “C-type” RNA viruses, and the “B-type” RNA mammary tumor viruses. Other DNA viruses, particularly the papovaviruses (47), are also demanding attention as oncogenic agents. Because of the relatively little information we have on the biology of the candidate tumor viruses, in vitro as well as in vivo, the risks inherent in vaccinating normal individuals with live attenuated virus are currently considerable. Killed virus, a possibility in spite of the problems of defining adequate bioassay systems for residual infectivity, is a possibility but shares with subviral fractions the potential of providing only short-term immunity. However, as immunological studies demonstrate “periods of high risk,” which are common in animal tumor systems and logically are present in tumors of childhood, the utility of such vaccines should not be completely ignored.

In view of the concerns regarding vaccination of normal individuals with potentially oncogenic materials, the use of antiviral vaccines at the present time would seem to be far more appropriate as part of an immunotherapy regimen in patients already afflicted with the disease. The accumulating evidence that persisting susceptibility, perhaps genetically related, maintains certain individuals at high risk to cancer indicates that “relapse” in a number of cases may indeed be reinduction of disease. Three examples of human tumor sites and is very responsive to retreatment with the BL, acute lymphocytic leukemia, and breast cancer. In BL, Ziegler has described 2 types of relapse, early and late, and has provided clinical evidence suggesting that late relapse is actually disease reinduction (59). His studies demonstrate that late relapse usually presents in sites other than the 1st tumor site and is very responsive to retreatment with the drugs used at disease onset, whereas the early relapse is resistant to the drugs used initially and local disease recurrence is the rule. Presuming that the late relapses (particularly those following unmaintained remissions of 6 months)

1 Presented at the symposium “Immunological Control of Virus-associated Tumors in Man: Prospects and Problems,” April 7 to 9, 1975, Bethesda, Md.
2 The abbreviations used are: BL, Burkitt’s lymphoma; EBV, Epstein-Barr virus; HSV-2, herpes simplex virus type 2.

...have the same etiology as the primary disease, control of the candidate etiological virus must be a matter of concern. A similar case can be made for breast cancer, where apparent cure of the disease is followed years later by a 2nd primary lesion in the other breast.

An even more dramatic example is acute leukemia, where apparent control of the disease through bone marrow transplantation has been thwarted by the transformation of the donor cells (56). One logical explanation given for the phenomenon is that the antitumor effect of the donor lymphocytes was successful but the putative human leukemia virus then infected the donor cells because there was no operating mechanism for controlling the virus. Thus, in those diseases where a viral etiology is likely, immunological control of the tumor may require control of the etiological agent as well. Since the persistence of the virus that initially caused the tumor may prevent the success of otherwise successful approaches, the addition of antiviral agents to the chemotherapy armamentarium offers interesting possibilities and will be discussed later in the Symposium (3).

In this meeting, we have chosen to concentrate on 4 tumors where we have information about specific candidate viruses. In one of these tumors, BL, there appear to be sufficient data linking the candidate etiological agent, EBV, and the disease, to move to the ethical considerations of how best to utilize the information in definitive clinical studies on etiology, prevention, and cure. Although it may appear that the evidence linking HSV-2 and cervical cancer remains tenacious, it is not premature to consider vaccines with this virus for 3 reasons: (a) in time, HSV-2 may prove to be the major etiological agent for cervical cancer; (b) herpes simplex undoubtedly is a human pathogen and a successful vaccine could be useful even if another etiological agent for cervical cancer is found; and (c) the accumulating immunological and epidemiological data on cervical cancer make this a reasonable time to reevaluate whether an antiviral vaccine has a role in this disease, regardless of whether HSV-2 ultimately is proven to be a contributing causative factor. Two other human tumors, breast cancer and acute leukemia, were chosen because of the clinical and experimental evidence for a viral etiology with the hope that tumor-specific and virus-related approaches can be utilized even without a definite etiological agent in hand. We intend to focus, therefore, on studies in these 4 virus-related tumors with the hope that the accumulating experimental data can be applied rapidly and effectively to the human cancer problem.

Because of the multiple problems associated with each virus-tumor system, each one presenting with unique epide-
miological as well as virological questions, the approaches that are relevant to one tumor may not pertain to another. Two of the diseases to be discussed in depth during this Symposium provide examples of this diversity: BL, where the candidate DNA virus is ubiquitous but infection is clearly influenced by environmental factors, and acute leukemia, where experimental evidence suggests that the oncogenic agent is most frequently transmitted vertically and derepression may be more important than external infection.

Burkitt’s Lymphoma

Problems in Prevention. The EBV should be considered in any discussion of possible human tumor viruses for the following reasons: (a) it is a bona fide human virus that has been demonstrated to be pathogenic for humans, producing a lymphoproliferative disease (infectious mononucleosis) at times resembling a cancer (23); (b) it is oncogenic for subhuman primates (12, 54); and (c) in 2 tumor systems where the virus has been etiologically implicated by seroepidemiologic studies, nasopharyngeal carcinoma and BL, the viral genome has been shown to be harbored within the neoplastic cells (28, 50, 51).

Elevated EBV titers have been reported in several lymphoproliferative tumors (22, 31, 32, 34, 37, 38), including Hodgkin’s disease and BL, where epidemiologic studies point strongly to an infectious etiological agent. While we will review the epidemiology of both of these lymphomas, the present evidence that EBV may be a human oncogenic agent rests most strongly on its association with African BL. Therefore, for the purposes of this program, which is oriented primarily toward control measures rather than problems in etiology, the lymphoma discussion will concentrate on the assumption that EBV is a major causative agent in BL.

The major impetus to a preventive vaccine for BL (or other EBV-associated tumors) has come from the success of a practical vaccine for Marek’s disease (4), a herpesvirus-induced lymphoma of chickens (10). This live vaccine, which will be discussed in detail later in the Symposium, is highly effective but the precise mechanism of action is still unknown. The impact of this vaccine on the poultry industry has been dramatic and, in looking for a human tumor where the principle of preventive vaccination could be effective, BL initially has some promising features. The identification of a high-risk group (children in holoendemic malaria areas) makes a limited field trial practical, and epidemiological evidence suggesting a short latent period (time and space clustering) enhances the likelihood that a vaccine may be effective. The protective effect of a vaccine, furthermore, is more easily demonstrated in a pediatric tumor where the young age peak would permit demonstration of effectiveness within 3 to 5 years.

In spite of these attractive features, however, the prospect of a preventive vaccine has raised enormous controversy which, in part, has led to the development of this meeting. BL is a rare tumor, even in the high-incidence areas and, since there are no good methods currently available to monitor the virulence of an attenuated vaccine or completeness of inactivation in a killed vaccine, the risk of vaccination may outweigh the potential benefit. Second, the same type of laboratory data (hybridization and fluorescence studies detecting EBV genome in the BL tumor cells) cited as strengthening the data etiologically linking EBV to BL also suggests that some cases may have different etiologies (33, 39, 51), thereby diminishing the possibility of an EBV vaccine on BL.

Other concerns include our lack of information about the epidemiology and biology of EBV infection, uncertainty as to whether there is only 1 EBV or whether there are a number of natural strains, and our lack of knowledge concerning the circumstances favoring an oncogenic effect. It has been demonstrated that EBV can be detected in virtually all parts of the world (57), but the age of infection is quite variable from region to region (11). Early studies attributed these differences to socioeconomic variations between the study groups, but recent comparative studies indicate that other factors may be playing an important role (11). Certain inferences about the mode of EBV transmission can be drawn from our knowledge about the epidemiology of infectious mononucleosis (13). It is now known that EBV is harbored in throat washings (9, 16), and seroepidemiologic studies indicate oral-fecal as well as an oral-oral mode of transmission. The possibility of a common source of infection, perhaps food or water supply, rather than person-to-person, may also be important, however.

The possibility that there are EBV strain differences has been suggested for some time (48), but only recently have studies begun to concentrate on the differing properties of various EBV isolates (33). The pursuit of such studies, still in their infancy, will be critical to determining whether different patterns of EBV-induced diseases (infectious mononucleosis, abacterial tonsillitis, mild upper respiratory infections, and possibly cancer) are the result of infection with different virus strains or the result of differing host response to the same agent. Certainly, it will be important to analyze immunological cross-reactivity between strains before a vaccine is to be considered.

Vaccines have effectively diminished the incidence of common childhood viral illnesses because of their impact on preventing disease associated with primary infection with a pathogenic virus. The possibility that EBV induces BL as a primary event even in a seronegative African child will be a precondition for a preventive vaccine to be utilized in BL. This question is now being investigated in a prospective study in the West Nile regions of Uganda, where 30,000 children are being bled and followed for the development of BL (17). It has been hypothesized that the time-space clustering seen in African BL results from episodes of EBV infection in unprotected children who are also suffering from the burden of malaria. If BL is found to occur only in individuals whose predisease sera are negative (and the current pattern of 100% seropositivity in post-BL serum is maintained), the prospective studies responsible for proving EBV to be a cause of IM (23, 44) will have been duplicated in a tumor system and will provide a great impetus to the idea of vaccination with EBV, at least in a high-risk (EBV seronegative) individual. It is also possible, of course, that very high EBV titers will be found in the predisease serum.
which could be subject to at least 2 interpretations, both of them tending to eliminate the possibility of vaccination. The 1st possibility is that repeated infection with EBV (probably associated with repeated assaults of malaria) is necessary for BL to occur. A 2nd possibility is that the high EBV titers are only a marker for susceptibility to BL which may be independent of a specific etiological role for EBV. This possibility is suggested by our family studies which have shown that normal individuals in families where there are multiple 1st degree relatives with cancer have significantly higher antibody titers to both the EBV viral capsid antigen and early antigen (35), even in families where the tumor type is very unlikely to be EBV-induced (sarcomas, pheochromocytomas, and thyroid carcinomas, for example). A 3rd possible explanation for high titers in predisease serum, that of a long latent period, is less likely in view of the explosive nature of the tumor and the presence of time-space clustering (42). It is possible, however, that it is a cofactor, not EBV, that provides these unique epidemiological features indicative of an infectious disease.

In view of the uncertainties regarding EBV despite a decade of study, other approaches to the prevention of BL also merit attention. The overwhelming evidence that holoendemic malaria plays a key role in causing BL (45) has led to the alternative possibility that malaria control rather than EBV control should be attempted in Africa. The dangers of complete control of malaria are as prominent as the difficulties, since the collapse of effective prevention would probably result in a rash of fatalities in the unprotected population. In contrast to elimination, malaria suppression is far less hazardous and certainly more practical. Because of the importance of BL as a model for other possible virus-induced tumors, the alternative approaches to prevention will be discussed in greater depth subsequently in the Symposium.

**Therapy.** Several aspects of BL make it a prime target for immunotherapy: it is highly antigenic (5); it appears to produce tumor-associated immunity in the host which at times is more important than conventional therapy (8); and chemotherapy effectively produces a minimal tumor burden. From the viral point of view, there are additional favorable aspects: the virus associated with the tumor is readily identified by specific techniques; there are useful viral assays of apparent prognostic value (19, 24, 55); and the concerns of inoculating a normal individual with an oncogenic virus are obviated, since the patient has already been infected, the purpose of the therapy being to give him a more useful immune response.

Initial attempts at specific immunotherapy of BL have not been encouraging. The observation of tumor regression after the receipt of immune serum (43) was followed by a controlled study that demonstrated no effect (14), thus suggesting that the initial report was comparable to the apparent spontaneous remissions documented by others (8). Because of the close relationship of EBV titers to the course of disease (19, 24, 55), serological monitoring of patients has been emphasized to date. The apparent failure of antibody to protect against tumor growth emphasizes the need for other approaches, such as the use of viral inhibitors and transfer factor. Transfer factor has not been utilized in BL, but attempts to treat another EBV-associated tumor, nasopharyngeal cancer, with transfer factor from patients with infectious mononucleosis may provide relevant data and will be discussed later (18).

**Acute Leukemia**

**Studies in Prevention.** The epidemiology of acute leukemia in the murine system indicates that the spontaneous form of the disease is frequently the result of virus transmitted via the gametes from parent to offspring (29). The presence of the viral genome in the newborn had initially been considered to lead to immunological tolerance and led to a general pessimism concerning immunological control of the disease, but it is now apparent that tolerance is not complete (46). Since subsequent speakers will review a great deal of what we now know about RNA tumor viruses in animals, I would only reemphasize that there is epidemiological evidence that at least some cases of animal (20) and human (6, 7, 41) leukemia are induced by environmental factors and, more important, recent clinical studies have clearly demonstrated that patients and close contacts are not tolerant to leukemia-associated antigens (1, 36, 52).

Attention to the possibility of a vaccine for prevention was generated by early studies of Fink et al. (15), demonstrating cross-reactivity between human leukemic cells and a laboratory strain of an RNA leukemia virus. A number of subsequent studies, biochemical as well as immunological, have continued to suggest that this cross-reactivity is real (21, 30, 40, 58), but the specific antigens involved in the cross-reaction have never been identified and there is some evidence that these antigens may not be specific for RNA viruses (40, 58). Attempts to develop a specific viral vaccine for human leukemia have not been generated because of the absence of these data on immunological specificity as well as serious concerns about the risks of a vaccine as compared to potential benefits. Recent reports, however, have raised the possibility of Bacillus Calmette-Guérin as a nonspecific preventive vaccine (53), and in this Symposium we will look at the possibilities for nonspecific as well as specific preventive measures for leukemia.

**Therapy.** Immunological management of acute leukemia will also be discussed later today, but it is worth commenting on several studies related to the viral aspects of the problem. Because of the apparent cross-reactivity between Rauscher leukemia virus and human leukemic cells, Hersh et al. (25, 26) attempted to define the parameters of immune response to Rauscher leukemia virus in humans as the basis for possible therapeutic studies in the future. By demonstrating that a good immune response could be mounted against prototype oncogenic RNA virus, Hersh’s studies (25, 26) laid the groundwork for the use of a viral vaccine in conjunction with chemoimmunotherapy. Additional studies on the immune response and a definition of which ones are important to a favorable outcome must still be carried out, but the approach of intercalating antiviral immune stimulants with other forms of therapy still seems to have a sound basis, particularly with the marrow transplantation findings noted above (56).

The use of allogeneic cells with enzymatic modification
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The success of preventive vaccines in apparently relevant virus-induced animal tumors has provided a hope for similar human tumor vaccines, but the risks of inoculating normal individuals with such vaccines may outweigh the potential benefits. The use of antiviral immunological and chemotherapeutic agents is quite appropriate, however, in attempting better control of the disease in patients already suffering from tumors suspected of having a viral etiology. The initiation of such studies may not only improve the cure rate of the cancer patients but will also undoubtedly add to our knowledge concerning the important immune responses needed to control these viruses. In this Symposium, we will reevaluate the feasibility of preventive vaccines and also review other applications of immunological techniques to the control of virus-associated tumors in man. As we will also discuss, knowledge of the epidemiology of each virus and the distinctive features inherent to each virus-virus system is critical, and it may be possible to develop an individual approach in the prevention as well as control of 1 tumor virus system that will provide us with useful data in others.

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