Viruses and Human Cancers: Challenges for Preventive Strategies

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Virus-associated human cancers provide unique opportunities for preventive strategies. The role of human papilloma viruses (HPV 16 and 18), hepatitis B virus (HBV), Epstein-Barr herpes virus (EBV), and retroviruses (human immunodeficiency virus [HIV] and human T-cell leukemia/lymphoma virus [HTLV]) in the development of common carcinomas and lymphomas represents a major cancer threat, particularly among individuals residing in developing countries, which account for 80% of the world’s population. Even though these viruses are not the sole etiological agents of these cancers (as would be the case for infectious diseases), different approaches can be implemented to significantly decrease the incidence of virus-associated malignancies. The first approach is vaccination, which is available for HBV and possibly soon for EBV. The long delay between primary viral infection and development of associated tumors as well as the cost involved with administering vaccinations detracts from the feasibility of such an approach within developing countries. The second approach is to increase efforts to detect pre-cancerous lesions or early tumors using immunovirological means. This would allow early diagnosis and better treatment. The third strategy is linked to the existence of disease susceptibility genes, and suggests that counseling be provided for individuals carrying these genes to encourage them to modify their lifestyles and other conditions associated with increased cancer risks (predictive oncology). Specific recommendations include: a) increase international studies that explore the causes of the large variations in prevalence of common cancers throughout the world; b) conduct interdisciplinary studies involving laboratory investigation and social sciences, which may suggest hypotheses that may then be tested experimentally; and c) promote more preventive and health enhancement strategies in addition to curative and replacement therapies. — Environ Health Perspect 103(Suppl 8):269-273 (1995)

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Origin and Cause of Human Cancers

To assess possible preventive strategies linked to the role of viruses in the causing human malignancies, one should reassess the role of oncogenic viruses in the framework of a multifactorial etiology of degenerative diseases. In the Pasteurian notion of the causative agents of infectious diseases, a microbe is the necessary and sufficient etiological factor. Since malignant diseases are noncontagious, it has been difficult to conceive that infectious agents could play a crucial role in their development. From the time that observations of avian leukemias and sarcomas were made by Ellerman and Bang, and Rous early this century to the present, a large body of data has accumulated that establishes a strong association between a number of exogenous oncogenic viruses and the development of tumors processes in many animal species and man. Doubts persist among many researchers regarding the role of viruses as etiological agents of certain human cancers. This is partially because for a given tumor such as Burkitt’s lymphoma (BL), the strength of the viral association may vary between geographical areas, and also because of the lack of a unifying concept for the molecular processes involved, both in experimental systems in man. These doubts reflect a lack of appreciation for the differences between the epidemiological concepts of origin and causation in the development of degenerative diseases.

The origin of a malignant disease lies in the irreversible and hereditary mutations occurring in expanding cellular clones that the organism neither recognizes as foreign nor rejects for other causes. On the other hand, it is well recognized that oncogenesis is a multistep process that takes years or even decades and involves successive gene disturbances and somatic mutations at the cellular level.

In epidemiology causation of degenerative diseases involves various factors that are linked to a significant increase in the incidence of the associated diseases or to a significant decrease after their eradication. Etiologic and risk factors—both of which are causative—have a great deal of practical value for the field of public health, since the removal of any of causal factors (either necessary and sufficient or significant risk factors) may potentially lead to partial or complete prevention of the disease.

Finally, it is well established that different oncogenic factors associated with tumor development do not add to but multiply their effect; for example, the combined effect of alcohol and tobacco on the pathogenesis of esophageal cancer in Brittany (2), and Epstein-Barr herpes virus (EBV) and malaria on the development of BL (3). Thus, it clearly is a priority to work to remove at least one of these factors, which has the potential of significantly decreasing the incidence of the associated tumor.

Epidemiologic Importance of Viral-associated Human Cancers

Doll and Peto (4), in their estimates regarding the avoidable risks of cancer in the United States, considered that about
10% of human tumors were associated with viruses. If one considers, however, the sizes of at-risk populations and not the proportion of virus-associated malignancies among all human tumors, a more accurate and urgent view is apparent.

Table 1 presents estimates for the primary virus-associated malignancies experienced in tropical and developing countries. Based on these data, viruses with onco-
genetic potential pose a threat to 80% of the world population, which is the percentage of individuals residing in the developing world. When establishing cancer research and treatment priorities, it must be remembered that approximately 5 million women in the world are affected by cervical carcinoma, which is associated with human papilloma virus (HPV). Hepatocellular carcinoma, which has been etiologically linked to hepatitis B virus, is responsible for the deaths of approximately 500,000 persons each year in tropical areas despite the existence of an efficient viral HBV vaccine for 15 years. This has primarily been a result of the inability of the Western World to produce an affordable vaccine for those with the greatest need. Nasopharyngeal carcinoma (NPC), which is a major cause of cancer-related death in Southeast Asia—more than 100,000 deaths a year are attributed to this cancer—exemplifies the potential effect that successful strategies for viral infection prevention can illicit.

Finally, tumors developing in acquired immunodeficiency disease syndrome (AIDS) patients, which consist of lymphomas, Kaposi’s sarcomas, and genital carcinomas, are increasing in relative frequency at the same time that better control of opportunistic infections is being achieved. This presents new challenges and suggests different priorities for biomedical researchers. High priority should be attached to comparative studies of these tumors among individuals with AIDS and those who do not have AIDS. In addition, the variations in incidence of these tumors by geographical area should be explored.

**EBV-associated Malignancies: A Model**

EBV is a ubiquitous gamma herpes virus that infects both epithelial cells of the oronasopharynx, where it replicates, and lymphoid B cells, where it remains latent in vivo, for the entire lifespan. The key pathogenic event for this ubiquitous, mostly innocuous, virus lies in the age and severity of the primary infection, which is mainly transmitted by saliva.

When primary infection occurs during adolescence, which is typical in industrialized countries, the predominant resulting clinical syndrome is infectious mononu-
clerosis. This disease is specific to individuals within high socioeconomic classes, and clinical recovery is dependent upon the efficiency of the T-cell-mediated immune response. Rare cases of malignant proliferations can result from primary infection by EBV such as non-Hodgkin’s lymphomas, which has been linked to a recessive gene on chromosome X—the Purtillo syndrome (5), or Hodgkin’s diseases, with an average latency period of 3 years after severe infectious mononucleosis. There is an increased relative risk of 3 to 4 among those who contract severe infectious mononucleosis compared with the risk of Hodgkin’s disease among the general population.

**Burkitt’s Lymphoma**

A potential, long-delayed consequence of severe primary infection by EBV is the African Burkitt’s lymphoma (BL), which is a high-grade malignant lymphoma of small, uniform, noncleared B lymphocytes. This lymphoma tests seropositive for CD19, CD20, and S1g surface markers. It is endemic to African children between 5 and 9 years of age, particularly in areas experiencing holo- or hyperendemic malaria.

To determine whether EBV was the primary or passenger virus in the tumorous B cells, we designed and conducted a major prospective study in Uganda between 1970 and 1978 that involved 42,000 children who were tested for EBV between 6 months and 2 years of age and followed up for 7 years. The analysis of pre- versus postdisease sera of the 16 children who developed BL showed that an early and severe primary EBV infection during the first months of life represented the key viral event for later development of the disease (6). It is highly probable that the primary cause of early EBV infection is a mother’s saliva and, to a lesser extent, her breast milk, since 65% of all African women of reproductive age shed infectious EB virus in their saliva, compared with 12% of women in the western world. The “feeding kiss” thus may be a major route of transmission for early EBV primary infection.

Another key environmental factor, namely holoendemic malaria, was first suspected by Burkitt (7). The experimental data of Moss et al. (8) showed that severe malaria burden specifically depressed T-cell cytotoxic clones recognizing the LYDMA epitope of the gp 340 viral glycoprotein at the surface of the infected B lymphocytes. The last oncogenic event in BL pathogenesis is caused by a chromosomal translocalation involving the long arm of chromosome 8 at the 8q24 locus, which is transposed either to chromosome 14, as in 80% of the cases, or to chromosome 2 or 22. This translocation exposes the c-myc protoonco-
genome to cis-activation by the immunoglobulin genes of the host chromosomes (9).

To determine the relative importance of each of these three independent factors—virus, parasite, and genetic event—we compared the incidence of the disease in three geographical areas, including: Europe, where 3% of the childhood tumors are attributed to BL and only 15% of these cases being EBV-associated; North Africa, where EBV infection occurs early but where malaria is not a burden; and East Africa where all three factors come into play. If the genetic activation of the c-myc oncogene were considered the necessary and
The molecular pathogenesis involving EBV genes in the transformation of nasopharyngeal epithelial cells remains an open question, and in contrast to the BL situation, EBV genes might act quite late in the development of NPC. As suggested by the IgA data mentioned before, local reactivity of latent EBV infection in nasopharyngeal mucosa precedes clinical symptoms by months, or even years. These specific secretory IgA may induce a shift in EBV lymphotropism toward epitheliotropism (17). If this is the case, the detection of IgA/VCA and IgA/EA antibodies originating in the nasopharynx would indicate emergence of pre-NPC tumorous clones. The majority of these clones, which are low-grade tumors, could be recognized and rejected by the organism, thus paralleling the loss of the IgA marker.

Need for Environmental Factors

The strong ethnic link between NPC and Cantonese Chinese, Maghrebian Arabs, and Greenland Eskimos, suggests a role for environmental factors in its development and based on anthropological studies, it was proposed that traditional preserved foods consumed by these three widely different ethnic groups could be involved (18). Chemical carcinogens, genotoxin, and EBV reactivators were detected in preserved meat, fish, and vegetables from the three areas. Aware that N-nitroso compounds can be formed in the stomach through nitrosation by bacterial nitrite reductase enzymes, a case-control study was implemented in south China using a urine N-proline test. The results of this study indicated that healthy individuals residing in high-risk NPC areas have significantly higher endogenous nitrosation rates than healthy persons residing in low-risk NPC areas (19). Furthermore, the dietary practices found among individuals in low-risk NPC areas included high levels of vitamin C, which was not true for the high-risk NPC areas.

At the same time, we screened for compounds that reactivated latent EBV within the traditional, preserved foods from south China, northern Africa and the Arctic. Dried and salted fish from China and traditional harissa (a spice mixture widely used in northern Africa) exhibited significant EBV replication-inducing activities. This discovery led to identification of an active fraction containing a lignin complex in harissa (20). It is possible that this compound is retained in the Rosenmuller recesses where, with the presence of nitrate-reductase bacteria, local reactivation of formerly latent EBV takes place.

Genetic Factors in NPC Development.

When sibships with multiple NPC cases were investigated in an international collaborative study involving China, Hong Kong, Singapore, and Malaysia, evidence pointed to the existence of a gene closely linked to the HLA region of chromosome 6 that conferred a greatly increased risk (relative risk = 21) for NPC (21). This NPC susceptibility gene has not yet been characterized, nor has its mode of action, which could potentially control the host immune response to EBV infection or the intermediate metabolism of the involved chemical carcinogens mentioned above. The deciphering of this interaction between environmental and host factors represents a primary challenge not only for preventing NPC but for the many human tumors in whose development both environmental and genetic factors interact.

Prevention of Virus-associated Cancers

The goal of epidemiologists is not just to test etiologic hypotheses but primarily to decrease disease incidence through preventive interventions. Early detection of virus-associated tumors such as the strategy described for NPC led to a dramatic improvement in the proportion of long-term survivors who had received successful radiotherapy. Individuals residing in Southeast Asia, who tend to favor preventive actions, are working to implement early detection efforts among the large at-risk population. Chinese individuals are culturally inclined to assume responsibility to avoid disease or improve their health. These individuals are willing to pay for preventive tests if they believe the tests are directly beneficial to their health. To this end, a cost/benefit analysis of early NPC detection should be completed. In contrast, northern African countries, whose populations tend not to focus on prevention regarding healthcare, follow the curative strategies favored by western countries.

Using antiviral vaccines to prevent virus-associated tumors is a controversial issue that is the topic of much research. The primary challenge to a conclusive evaluation of the efficacy of vaccination is posed by the long latency period that elapses between primary infection and tumor development, which makes the effects of this preventive intervention difficult to assess. The second difficulty is the cost involved in large-scale vaccination.
The challenges encountered when administering a large-scale HBV vaccination program within a developing country are exemplary. The question of the necessity of an EBV vaccine has been repeatedly addressed, and its value in preventing diseases linked to primary EBV infections is well-accepted. This would involve prevention of severe infectious mononucleosis, EBV-associated Hodgkin's diseases, and X-linked lymphoproliferative syndromes that are, as noted above, linked to severe primary EBV infection. The value of vaccination to reduce EBV-associated lymphomas emerging among individuals living with AIDS is more difficult to assess, since vaccination would occur in latently infected individuals. The chance to boost the EBV specific cell-mediated immunity in these conditions merits investigation.

Administering large-scale EBV vaccines to prevent African Burkitt's lymphoma is theoretically ideal. In view of the present circumstances, however, where 30% of African newborns die before 5 years of age from severe malaria or measles and the proportion of AIDS-related deaths is dramatically increasing, a vaccination that would eliminate a tumor that occurs in only 1 of 1000 surviving children appears to be a low priority.

Regarding NPC, the primary question is: Would a vaccination administered during the first few months of life—before primary EBV infection—be able to induce a long-term humoral and cell-mediated immunity sufficient to protect individuals for decades? It must be remembered that these individuals would be environmentally at-risk to develop NPC 25 to 40 years after being vaccinated. However, administering therapeutic EBV vaccines among IgA/VCA-seropositive individuals who are at immediate risk for developing NPC merits consideration.

Conclusions and Proposals
The above examples of virus-associated malignancies and our increasing knowledge of the multifactorial, multistep carcinogenic process in humans have practical applications for both fundamental and applied cancer research. I suggest three approaches that could improve our efficiency in reducing cancer morbidity and mortality during the coming years.

The first is to widen epidemiologic studies by investigating more thoroughly the large variability of specific cancers throughout the world and to explore the possible causes of these variations. American taxpayers wish to concentrate research efforts on the most common cancers in the United States and this is understandable. These cancers (breast, colon, and prostate), however, vary widely in incidence throughout the world, and comparative investigation of the putative oncogenic environmental factors in high- and low-risk areas for a given cancer could lead to dramatic advances in knowledge of their etiology. In industrialized western countries, these malignancies are epidemiologically linked, as evidenced by the similar increases in the incidence of all three cancers experienced among Japanese immigrants to U.S. territories. My first proposal is to develop internationally collaborative epidemiologic studies in various locations throughout the world to study the primary cancers among U.S. citizens, including those cancers associated with HIV-AIDS, which also exhibit great geographic variability.

The second recommendation is to utilize a more interdisciplinary approach when investigating common cancers. The results obtained regarding the etiology of NPC were achieved by collaboration of a food-habit anthropologist and a chemical carcinogens group. This interesting case required examination of common factors among ethnic groups at high risk of developing NPC instead of differences between cases and controls. It therefore is becoming increasingly evident that the complexity of interactions between host genetic factors and environmental carcinogens (i.e., biological, chemical, or physical factors) can only be properly investigated through interdisciplinary studies involving, in addition to epidemiologists and laboratory scientists, anthropologists, who investigate how people live, eat, drink, love, and die. Practical incentives that promote interdisciplinary approaches to investigating cancer etiologies should be included within NCI grant-related activities.

My third proposal is to promote further shifts in the emphasis of the nation's approach to health care. In the last 15 years, U.S. citizens have begun to accept that a preventive approach to health care is complementary to and to some extent more cost efficient than curative medicine and replacement surgery. In addition, the exponential development of molecular genetics, which is permitting the characterization of disease susceptibility genes, is opening the way for a predictive oncology that may seal the cultural shift toward prevention-oriented medical practices.

Considering the prevalent role of lifestyles, primarily dietary habits, in the development of common cancers, the search for specific cancer susceptibility genes should further cancer prevention in two ways. The first, which has already been implemented in a number of specific genetic counseling centers in the United States and Europe, is to suggest to carriers of specific disease susceptibility genes that they participate in screening activities to ensure early detection of preterminal or tumorous lesions, which may permit curative treatment (preclinical medicine). The second is to counsel individuals with specific genetic traits to modify their lifestyles, including food habits, to decrease their cancer-associated risks. Because the interactions of various carcinogenic factors are multiplicative in their effect and not simply additive, this preventive attitude could have a significant effect on several common cancers, either by decreasing their incidence or by delaying significantly the clinical onset of these diseases—perhaps by 7 to 21 years, according to Doll and Peto (4).

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