Epstein-Barr Virus—Is It Time To Develop a Vaccine Program?

Whatever may be thought of the magnitude of effort and expense devoted to research on tumor viruses or the various directions in which particular thrusts are encouraged from time to time, there can be no controversy over the ultimate goal. Quite simply, the goal must be to acquire sufficient knowledge of viral oncology ultimately to eliminate virus-induced malignant tumors of man.

The main steps toward this objective were clearly established when the Special Virus-Leukemia Program (which later became the Special Virus-Cancer Program) was initiated in 1964. Viral agents were to be sought in tumors and their constant association with human cancer was to be established. Efforts were to be made to determine whether at least one human cancer had such an associated virus as cause, and if so, an effective antivirus vaccine was to be developed for the control of the tumor (1, 2).

In recent years, several suspected human tumor viruses have been identified; some have survived scrutiny and are currently under investigation. But with any such agent, direct proof of a role in the cause of human malignancy is likely to be impossible to obtain without violating ethical barriers, and only indirect and circumstantial evidence will be available. The question will, therefore, always remain as to the value of accumulating yet more and more information on the association of an agent with a given human tumor, since this will never provide a final, definitive answer. At some stage a more dynamic approach must be devised, and it may well be that this should be undertaken soon.

Of the viruses currently suspected of involvement in the etiology of human cancer, it is not unreasonable to say that the Epstein-Barr virus (EBV), discovered in relation to Burkitt’s lymphoma (3), is the only really convincing candidate at present. The reasons are that EBV fulfills some of the major requirements envisaged at the start of the Special Virus-Leukemia Program: It is an unusual new human virus, it shows a constant close association with two specific types of human malignant tumors, and its biologic behavior includes features not unlike those of known oncogenic animal viruses. Thus properly authenticated cases of African Burkitt’s lymphoma and nasopharyngeal carcinoma (NPC) only occur in individuals infected by the virus; the viral DNA is present in the tumor cells and determines the expression of them in virus-coded neoantigens. Therefore, it is not surprising that virus production can be activated in some of the tumor cells by various laboratory procedures. The virus is a powerful stimulator of lymphoproliferation in vivo as the cause of Paul-Bunnell-positive infectious mononucleosis (IM); it is a powerful stimulator of lymphoproliferation in vitro, conferring the property of continuous growth on normal human B lymphocytes with many changes analogous to malignant transforma-

tion; and it is carcinogenic experimentally in vivo, causing malignant lymphoma in inoculated South American subhuman primates, with Koch’s postulates fulfilled in the system. Animal herpesviruses behaving similarly to EBV cause malignant lymphoma or carcinoma in natural or experimental hosts. Recent publications have documented each of these points (4–8), but, as expected, this impressive evidence suggesting that EBV plays some part in the etiology of African Burkitt’s lymphoma and NPC (with whatever cofactors may be involved) is wholly circumstantial and inferential.

However, it appears that presently we know more than enough to incriminate EBV as a prime suspect for the role of human tumor virus, and the question of direct proof must now seriously be considered. As has already been suggested (9), the only way to resolve this difficulty would be the development of an experimental vaccine to be used not only with the long-term hope of controlling human malignancies, but in the short term as a means of establishing the carcinogenicity of EBV.

The difficulties in preparing and administering to human populations a safe and effective vaccine for a suspected tumor virus are obvious, but recent work indicates that much less may be involved than has sometimes been feared in the past. There is also the question whether the considerable efforts that such a program would need are worthwhile. Apart from the scientific objective of showing that at least one human tumor has a viral etiology, there are also important practical reasons for perservering with EBV in this respect. Although Burkitt’s lymphoma occurs frequently in limited areas and does not involve particularly large numbers even in these areas (10), an effective vaccine to EBV protecting against Burkitt’s lymphoma would demonstrate some causative role for the agent in this tumor and thus would immensely strengthen the likelihood that EBV is likewise an etiologic agent in NPC. A high incidence of NPC is also limited in its geographic distribution, but here the limitation is racial, being confined to Southern Chinese people, and there are very large numbers of these in various parts of the world. Thus although Burkitt’s lymphoma may be a minor medical problem, NPC is considerably important in terms of world health, since it is the commonest cancer of adult males and the second commonest cancer of adult females of Southern Chinese

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Editor’s note: Periodically, the Journal publishes solicited guest editorials as a means of transmitting to investigators in cancer research the essence of current work in a special field of study. The Board of Editors welcomes suggestions for future editorials that succinctly summarize current work toward a clearly defined hypothesis regarding the causes or cure of cancer.
origin (11) who, with related races, form about one-third of the population of the world (12).

Any scheme for an anti-EBV vaccine will need to be performed in two steps: 1) a small-scale pilot experiment to prove the carcinogenicity of the virus in relation to African Burkitt's lymphoma; and 2) a more complicated, long-term project, the wide-scale vaccine control, not only of Burkitt's lymphoma, but more importantly of NPC with its great significance in terms of numbers involved. There are special advantages afforded by the EBV and African Burkitt's lymphoma system, as pointed out elsewhere (4). Since EBV causes IM, the antiviral efficacy of any vaccine could be tested by its ability to protect those at risk from natural primary EBV infection accompanied by this disease. In addition, there are well-recognized areas of high endemicity of African Burkitt's lymphoma, where the effect of a vaccine on tumor incidence could be tested. Finally, since African Burkitt's lymphoma is a disease with a peak incidence in children around the age of 6, it should be possible to vaccinate all 0-to-1-year-old members of a population in an endemic area and to judge the effect on tumor development within 5-10 years; thus an answer would be obtained far more rapidly than possible with most other human tumors which occur mainly late in life.

Concerning the practicality of vaccination, control of a naturally occurring, herpesvirus-induced malignant tumor by an antivirus vaccine was demonstrated 6 years ago when live, apathogenic herpesvirus vaccines were introduced to protect chickens against the development of Marek's lymphomas (13, 14). However, the applicability of a live virus vaccine to the human situation is obviously doubtful because it is impossible to administer a suspected tumor-inducing virus, however attenuated, to man. Even a conventionally inactivated virus of this kind would raise strong objections for use as a human vaccine because of the problem of proving total inactivation and the possibility that traces of viral nucleic acid in such a preparation could induce malignant transformation. However, further progress with vaccines against oncogenic, animal herpesviruses has indicated the ways in which such difficulties can be overcome. It is now known that chickens are significantly protected against lymphomas of Marek's disease by vaccines free of viral nucleic acid. Lesnick and Ross (15) reported success with a vaccine consisting only of soluble viral antigens extracted from Marek's virus-infected tissue culture cells by treatment with nonionic detergent; other workers (16) have used highly purified plasma membranes from similar virus-infected cells as a vaccine and have thereby reduced the mortality rates from Marek's lymphomas by 94% when the vaccinated chickens were subsequently challenged with virulent virus. The whole question was recently moved closer to man when Laufs and Steinke (17) showed that subhuman primates can also be successfully protected against a herpesvirus-induced malignant lymphoma. The researchers used heat and formaldehyde to inactivate Herpesvirus saimiri, which is otherwise carcinogenic in marmosets (19), and  complement-fixation tests have shown that the inactivated vaccine maintains considerable, specific viral antigenicity after these treatments.

Numerous cottontop marmosets have been immunized with the vaccine, remained well, and developed high titers of neutralizing and complement-fixing antibodies to the virus. When last reported (17), 22 immunized animals had been challenged with a large tumor-inducing dose of virulent H. saimiri and continued alive and well for many months, whereas inoculated control animals died of malignant lymphoma within 52 days. There seems no reason to doubt that nucleic acid-free vaccines will soon be developed for H. saimiri like those already used against the herpesvirus causing Marek’s lymphomas in chickens. Development of a viral, nucleic acid-free vaccine for EBV along similar lines will clearly meet many of the previous ethical objections to the concept of a vaccine for this suspected human tumor virus, particularly if purified antigens are used.

The methodology for preparation of nucleic acid-free, herpesvirus vaccines to control animal tumors is rapidly progressing, and the application of the necessary techniques to EBV is unlikely to present significant difficulties. It has sometimes been argued that the absence of a fully permissive cell for the replication of EBV presents an insuperable problem for vaccine production, but this argument misses the whole point: with this system, virus production is both unnecessary and undesirable. There are available appropriate, continuous human lymphoid cell lines that do not replicate EBV but do have virus-determined membrane antigens expressed on the cell surface, and it seems that antibodies to these membrane antigens also have virus-neutralizing activity (19–22). No difficulty or special cost is involved in growing such cells on a large scale, and techniques both for preparing cell membranes from human lymphoid cells and for purifying antigens from the isolated membranes are well tried (23–26).

Two main safety factors are of fundamental importance in the administration of such a vaccine to man. First, the total absence of viral DNA must be confirmed in the material to be inoculated; this is probably much less difficult than the more complicated problem presented by the safety testing of polio vaccines 20 years ago. Second, and perhaps more important, safety also demands demonstration that the vaccine does not engender immunopathologic complications after administration. Although this hazard seems unlikely if purified membrane antigens are used, the problem of enhancement must be considered. It could be argued that use of a killed vaccine for immunization of a population at risk for the development of Burkitt’s lymphoma would be justified only if immunity were maintained by booster doses throughout life, since a fall in immunity might permit EBV infection with the development of blocking factors capable of enhancing the growth of any malignantly transformed Burkitt’s lymphoma cells that subsequently emerged. However, it could equally be that a high secondary antibody response to infection may result from previous but lapsed vaccination, may directly limit the growth of such tumor cells, and may thus be beneficial. In any event, these questions can now be tested experimentally with the availability of both subhuman primates susceptible to tumor induction by EBV (7, 8) and of the H. saimiri model with the use of killed vaccine in marmosets (17, 18).

Susceptible subhuman primates are also necessary for the essential in vivo laboratory testing of the protective efficacy of a vaccine. Once protection has been demonstrated satisfactorily in animals and the safety problems have been resolved, efficacy of the vaccine in preventing EBV infection in man is the next step; this can be investigated in the context of EBV seronegative young people at risk for primary infection in the age group in which
EBV IS IT TIME TO DEVELOP A VACCINE PROGRAM?

EBV is a necessary etiologic agent in the induction of NPC. Vaccination to protect against this malignant tumor of later life poses considerable problems in the maintenance of immunity to EBV over many years, and modifications would be required in the type of vaccine to be used. The difficulties here should not be minimized, but since NPC is the commonest tumor among a substantial section of the world population, no effort should be spared if consideration for another large and equally significant area of the world.

For a field trial on children in an area where Burkitt's lymphoma is highly endemic, it is doubtful whether a membrane vaccine could induce sufficiently long-lasting immunity, and revaccination would be required to maintain protection from the earliest postnatal months to the age of 5 or 6 years, when African Burkitt's lymphoma has its peak incidence. However, even in remote and undeveloped regions, it is perfectly possible to successfully mount complicated programs involving considerable numbers. The West Nile District of Uganda Project currently functioning under the auspices of the International Agency for Research on Cancer (30) is a Burkitt's lymphoma-related example of such a program that depends on relatively simple financial and logistic support. Blanket vaccination of an appropriate postnatal age cohort in an area such as the West Nile District is not likely to prove any more complicated even with the necessity for subsequent booster doses. The relatively small West Nile District has a dense population with a sufficiently high tumor incidence (27) for a drop referable to an EBV-independent vaccination program to be recognizable. It would thus be an appropriate place to test such a vaccination scheme, as would many other places in which similar high incidences of Burkitt's lymphoma are also known.

Should the results of such a vaccine program indicate that EBV is causally related to African Burkitt's lymphoma even in association with cofactors, the case will be immensely strengthened for its consideration as a likely etiologic agent in the induction of NPC. Vaccination to protect against this malignant tumor of later life poses considerable problems in the maintenance of immunity to EBV over many years, and modifications would be required in the type of vaccine to be used. The difficulties here should not be minimized, but since NPC is the commonest tumor among a substantial section of the world population, no effort should be spared if control seems possible. If such control of an exceptionally common tumor among Western populations were in sight, support would be forthcoming; the effort should surely be similarly worth consideration for another large and equally significant area of the world. If the recent developments and possibilities outlined above are considered, it does not seem unrealistic to suggest that planning for the investigation of the many problems ahead would not be premature at the present time.

REFERENCES


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