Implications of a Vaccine for the Prevention of Epstein-Barr Virus Infection: Ethical and Logistic Considerations

M. A. Epstein

Department of Pathology, University of Bristol, The Medical School, University Walk, Bristol BS8 1TD, England

Summary

Reasons are given for considering that there is sufficiently substantial indirect and circumstantial evidence linking Epstein-Barr (EB) virus to African Burkitt's lymphoma (BL) and nasopharyngeal carcinoma to call for a dynamic new approach to establish a causal role for the virus in these human cancers. It would seem that the only way to do this would be to develop a vaccine, vaccinate a population at risk in a high-tumor-incidence area, and subsequently follow the population for a consequential decrease in tumor incidence. Recent developments in the control of animal herpesvirus-induced malignant tumors by vaccines free of viral nucleic acid make it possible to envisage that a similar vaccine could be developed against EB virus without undue difficulty. Experiments showing the tumor-inducing ability of EB virus in South American subhuman primates have provided an in vivo laboratory system in which to test the safety and efficacy of the vaccine. Trial of the vaccine in human populations could be carried out by testing its ability to protect those at risk from primary EB virus infection accompanied by infectious mononucleosis.

Although in world terms BL is not a major health problem, nevertheless African BL provides uniquely favorable conditions in which to test for a causative role for EB virus: high incidence areas are known, the peak tumor incidence is at the age of 5 or 6, and the effects of vaccination on tumor incidence could be assessed within a decade.

Should a carcinogenic role for EB virus be demonstrated in African BL, a much longer term program would be called for to extend the vaccine control of infection to areas where EB virus is implicated in the induction of nasopharyngeal carcinoma. Although a high incidence of this tumor is confined to populations of Southern Chinese origin, the very large numbers of such people and the frequency of the tumor among them make this a substantial world health problem and, therefore, worth the cost and effort necessary to develop a vaccine giving life-long immunity and to conduct a program that will take more than a generation to give positive results.

Introduction

It is now 11 years since the Special Virus Leukemia Program (later expanded to the Special Virus Cancer Program) was first set up, and from the outset 2 main objectives were clearly defined: (a) to determine whether at least 1 human cancer is caused by an oncogenic virus; and, if so (b) to develop an effective vaccine for the control of such a tumor. As a preliminary to these objectives, unknown viral agents needed to be identified and their association with human cancer had to be established (2, 25). At the time, this project was viewed with considerable scepticism by many leading workers in the cancer and virus fields, but looking back at the impressive progress made in tumor virology over the past decade, the farsightedness of the concept is now evident.

Important advances have been made in our knowledge of animal oncogenic viruses and certain suspected human tumor viruses have been identified, but it is fair to say that EB virus, first discovered in relation to BL (11), has steadily emerged as the leading and only really convincing candidate for the role of the 1st cancer-causing virus of man. The reasons for this arise from its consistent association with 2 human tumors and may be summarized thus: (a) authenti
cated cases of African BL and NPC occur only in individuals infected by the virus; (b) the viral DNA is present in all the tumor cells and determines the expression in them of virus-coded neoantigens; not surprisingly therefore, at least with BL, virus production is activated in some of the tumor cells when they are placed in tissue culture; (c) the virus is a powerful stimulator of lymphoproliferation in vivo as the cause of Paul-Bunnell-positive IM; (d) the virus is a powerful stimulator of lymphoproliferation in vitro, i.e., it confers the property of continuous growth on human B lymphocytes together with many changes reminiscent of malignant transformation; (e) the virus is carcinogenic experimentally in vivo, causing malignant lymphoma when inoculated in South American subhuman primates in such a manner that it has been possible to fulfill Koch's postulates; and (f) animal herpesviruses behaving similarly to EB virus cause malignant lymphoma or carcinoma in natural or experimental hosts. These points have been discussed and documented elsewhere (9, 12, 15, 17, 29).

In view of the foregoing and with the original objectives of the Special Virus Leukemia Program in mind, the question arises as to whether it is now time to consider the desirability and problems of a vaccine program for EB virus that, being horizontally transmitted, is amenable to such an approach.

1 Presented at the symposium "Immunological Control Virus-associated Tumors in Man: Prospects and Problems," April 7 to 9, 1975, Bethesda, Md.

2 The abbreviations used are: EB, Epstein-Barr; BL, Burkitt's lymphoma; NPC, nasopharyngeal carcinoma; IM, infectious mononucleosis.
The Question of Timing

The impressive evidence suggesting some sort of etiological role for EB virus in African BL and NPC with whatever cofactors may be involved is, of course, wholly circumstantial and inferential, and it is difficult to see how direct proof of carcinogenicity in man can be obtained without violating ethical barriers. It must be decided, therefore, whether the accumulation of yet more and more indirect evidence for the association of the agent with human cancer is likely to be of value since information of this type can never give a final definitive answer. At some stage a more dynamic approach must be decided upon. It would appear that at the present time enough is known to incriminate EB virus and the difficulty of directly proving its carcinogenicity must be resolved. As has already been pointed out (7), the only way to do this would be to develop an experimental vaccine, carry out a trial pilot vaccination program in an area of high tumor incidence, and follow this with prospective surveillance to detect any decrease in the expected number of cases consequent on prevention of infection. No other approach to establishing some direct causal relationship between EB virus and a human tumor would appear feasible.

Economic Considerations

The difficulties in preparing and administering to human populations a safe and effective vaccine for a suspected carcinogenic virus are obvious and would call for considerable efforts and financial backing. Since BL occurs frequently only in rather limited areas and even in these does not involve very large numbers (3), it may be questioned whether the effort and expense required can be justified. In any case the high incidence areas are just those with many more pressing medical and community health problems. However, an effective vaccine to EB virus protecting against BL would clearly demonstrate some causative role for the agent in this human cancer and would, thus, immensely strengthen the likelihood that the virus was likewise an etiological agent in NPC. Although a high incidence of NPC is also limited in its geographical distribution, this limitation is largely confined to people of Southern Chinese origin, and there are very large numbers of such people. Thus, although BL may be a relatively minor medical problem, NPC is certainly of very considerable importance in terms of world health since it is the commonest cancer of adult males and the 2nd commonest of adult females among the Southern Chinese (28), who form an important segment of the world population. Besides the high-incidence areas, regions with a significant moderate incidence have been recognized more recently.

As to effort and expense, recent developments with animal oncogenic herpesvirus vaccines suggest that much less may be involved than has sometimes been feared in the past. These developments have already been commented upon (8) and are discussed further below.

Planning Considerations

The planning of a vaccine for EB virus must envisage 2 steps: (a) a small-scale scheme to prove the carcinogenicity of the virus using African BL as the test tumor since there are numerous special advantages with this disease; (b) vaccine control on a much wider scale not only of BL but also of NPC with its much greater significance in terms of numbers involved.

As has been pointed out (9), EB virus and African BL provide a system with 3 unique advantages. First, since EB virus causes IM (10), the antiviral efficacy of any vaccine can be tested by its ability to protect those at risk from this disease. Second, there are well-recognized localized areas of high endemicity of African BL where the effect of such a vaccine on tumor incidence could be relatively easily tried out (3). Third, since African BL is a disease with a peak incidence around the age of 6 (3), it should be possible over a given period to vaccinate all members of a population aged 0 to 6 months in an endemic area and to judge the effect of this on tumor development within 5 to 10 years; such a rapid answer could not be obtained with most other human tumors that occur mainly late in life.

Ethical Considerations

The practicability of controlling a naturally occurring herpesvirus-induced malignant tumor by an antiviral vaccine was demonstrated in 1969 with the introduction of live apathogenic herpesvirus vaccines capable of giving chickens almost complete protection against Marek's lymphomas (4, 21). The importance of this step forward was widely recognized both because of its economic significance and because it provided the 1st example of a naturally occurring malignant tumor to be controlled in this way. However, the applicability of a live viral vaccine to the ultimate control of those human tumors suspected of having a herpesvirus cause was clearly slight because of the impossibility of administering to man a suspected tumor-inducing virus, however attenuated. Indeed, it is unlikely that even a conventionally inactivated virus of this kind would be useable as a human vaccine because of the difficulty of proving total inactivation and the possibility that traces of the viral DNA in such a preparation might be capable of bringing about malignant transformation. Recently, however, further progress with oncogenic animal herpesvirus vaccines has begun to indicate the ways in which these difficulties could be overcome. It is now known that chickens can be significantly protected against Marek's lymphoma by vaccines free of virus nucleic acid consisting either of soluble viral antigens extracted from Marek's virus-infected tissue culture cells (19), or of highly purified plasma membranes from such cells (16).

There is no reason to doubt that similar nucleic acid-free vaccines to other oncogenic herpesviruses can be developed. A killed vaccine to Herpesvirus saimiri has already been shown successfully to protect immunized marmosets against subsequent challenge with huge tumor-inducing doses of the virus (18), and it should not be difficult to develop H. saimiri vaccines free of viral nucleic acid. This virus and the susceptible marmosets in which it causes tumors (20) thus provide a satisfactory laboratory model system for studying the vaccine control of herpesvirus-induced malignant lymphoma in primates.
Once a viral nucleic acid-free vaccine for EB virus is developed in a manner similar to the above, many of the ethical objections raised in the past to the concept of a vaccine for a suspected human tumor virus will have been met, particularly if purified antigens are used for the immunization.

**Logistic Considerations**

With the methodology rapidly being worked out for the preparation of nucleic acid-free herpesvirus vaccines to control animal tumors, the application of the necessary techniques to EB virus is unlikely to present significant difficulties. Although a fully permissive cell system for the replication of EB virus has not thus far been discovered, such a system is not in fact necessary for EB virus vaccine production. Appropriate continuous human lymphoid cell lines are available that do not replicate EB virus but that do have virus-determined membrane antigens expressed on the cell surface and there is growing evidence that antibodies to these membrane antigens also have virus-neutralizing activity (6, 13, 22, 23). There is no problem in growing such cells on a large scale and the cost of this is not especially great. In addition, there are well-tried techniques both for preparing cell membranes from human lymphoid cells and for purifying antigens from the isolated membranes (5, 26, 27, 30), and again neither of these steps is unduly expensive.

With regard to the preliminary testing of a cell membrane vaccine against EB virus, laboratory studies in an in vivo system have become possible since the oncogenic potential of the virus was demonstrated recently in South American subhuman primates (12, 29), and the position should soon become analogous to that with *H. saimiri* in such animals.

Both from the ethical and the logistic point of view the main safety factor relates to verification of the absence of viral DNA in material to be used as a vaccine; but by comparison with the immense efforts put into the much more complicated problem of the safety testing of polio vaccines 20 years ago, this elimination of EB viral DNA presents much less difficulty. Safety also demands the demonstration that vaccine material does not engender immunopathological complications after administration. Although this seems unlikely if purified membrane antigens are used, the question can be settled experimentally with susceptible subhuman primates using both *H. saimiri* as a model and the EB virus system itself.

The existence of susceptible subhuman primates also provides the means for the essential in vivo laboratory testing of the protective efficacy of a vaccine. Once protection has been demonstrated satisfactorily in animals, the efficacy of the vaccine in preventing EB virus infection in man is the next step, and this could be investigated in the context of EB virus-seronegative young people at risk for primary infection in the age group where this is accompanied by IM. Groups of such populations at risk come into Western Universities and other training establishments each year, and an example of the completeness with which a particular group can be followed has recently been provided by the elegant Yale prospective study of EB virus infection in West Point cadets (14).

Assuming then that preparation of a membrane vaccine, laboratory testing of such a vaccine, and authentication of its effectiveness in animals and by prevention of IM in EB virus-negative individuals can be successfully achieved, there remains the final important step of using the vaccine in a field trial to show that prevention of EB virus infection abolishes the occurrence of BL in a high-incidence area. This experiment is crucial for demonstrating some sort of causative role for the virus in African BL and thus for showing whether or not subsequent development and large-scale use of an EB viral vaccine to control NPC should be undertaken.

Ethical objections to the use of such a vaccine on African children will have been removed by the previous clinical trials involving Western student groups, and only the logistic problem of using the vaccine in Africa will remain. It is doubtful whether a membrane vaccine could induce immunity lasting more than a few years and for a field trial, therefore, more than 1 vaccination will be required to maintain protection in individuals from the earliest months to age of 5 or 6 years when African BL has its peak incidence. However, even in unstable and remote regions of Africa it is possible to successfully mount complicated health studies involving considerable numbers. The West Nile District of Uganda Project, currently going forward under the auspices of the International Agency for Research on Cancer (1), indicates that quite sophisticated investigations can be achieved in such a remote situation with relatively simple financial and logistic support, and blanket vaccination of an appropriate postnatal age cohort in an area such as the West Nile District is likely to prove far less complicated, even taking into account the necessity for subsequent booster doses. Although the West Nile District of Uganda measures only about 110 x 50 miles, the population is dense, and the tumor incidence is high enough for it to be an appropriate test area; in a recent 5-year period, 40 authenticated cases of BL were recorded (24). It should not be difficult to recognize a drop in such an incidence level referable to an antecedent vaccination program, and there are many other areas with a similar high incidence of BL.

Should the results of a vaccine program such as that described above indicate that EB virus is causally related to African BL even with associated geographical cofactors, the case will be irresistible for seeking to extend the investigation to the control of EB virus tumor induction in NPC. However, this is a malignant tumor of later life and the problem would have to be faced of maintaining immunity to EB virus over very many years. It is doubtful whether this could be done by means of a straightforward membrane vaccine but, on the other hand, the incidence of the disease in terms of world health is very considerable and the necessary effort to develop acceptable alternatives would clearly be worthwhile.

**Conclusion**

An antiviral vaccine to control BL in African children appears to have become a practicable possibility and is likely to give definitive answers within a decade. Extension of such a vaccine program to the control of NPC could well
grow from this, but it is now only a distant hope and it will take more than a generation to obtain results.

References

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M. A. Epstein


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