Preventive Vaccination against *Herpesvirus saimiri*-induced Neoplasia

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Summary

In this paper we discuss the use of *Herpesvirus saimiri* as a model for the development of vaccines against herpesvirus-induced neoplasia in primates. Attempts at protection against the oncogenicity of *H. saimiri* have centered on the inactivation of virus by heat and formalin, the production of temperature-sensitive *H. saimiri* mutants, and the attenuation of the virus. Each of these approaches has provided information of use in the development of vaccines that may possibly be used in man.

Introduction

Although investigators have used rodents and avian species for studies relating to viral and chemical carcinogenesis, nonhuman primates offer potential advantages for a variety of reasons (7). Primates bear a close phylogenetic relationship to man. They have similar serum proteins, α-fetoprotein, and carcinoembryonic antigen; their metabolic pathways are often closer to man's than are those of rodents or birds. Indigenous simian agents, such as B virus (38), may bear a close pathogenetic and immunological relationship to their human counterparts. Longer term studies of carcinogenesis are possible in primates because of their greater lifespan, and their larger size allows more versatility in the design and execution of experiments. Finally, nonhuman primates have been used for the evaluation of antineoplastic agents (6, 36) and in the development and testing of vaccines against such pathogens as polioviruses (27, 33). Although the availability of these animals is becoming a problem, they would be very important in the study of humoral and cellular immune responses in relationship to viral oncogenesis and in the development of vaccines against oncogenic viruses (41). Due to the considerable emphasis and interest for the past few years in using HVS as a model for EBV (4), our remarks in this communication are restricted to the data that indicate that this virus is a suitable candidate for vaccine studies with those human herpesviruses that are strongly implicated in the etiology of certain cancers.

The development of a preventative vaccine for HVS-induced lymphoma is important for at least 2 reasons. The 1st, and more immediately practical of these, is for the protection of primate species which are susceptible to the oncogenic effects of HVS and which may come into contact with squirrel monkeys, many of which carry the virus and transmit it horizontally (3, 4, 17, 23, 31). The 2nd and more speculative reason deals with the use of the HVS tumor-vaccine system as a model for efforts designed ultimately to protect humans from the neoplastic sequelae of human herpesviruses such as EBV (11, 14, 15, 22, 26, 32) and *Herpes simplex* virus type 2 (8, 9, 34, 37), which may cause cancer. Work dealing with a human model is more exacting, because the nucleic acid of a potentially oncogenic virus should not be introduced into a human; work with a strictly animal system offers a wider range of possibilities and is a good place to begin. Research with HVS vaccines may ultimately come to resemble that for Marek's disease, for which a variety of live-virus vaccines (10, 35), as well as vaccines not containing virus nucleic acid, have been developed (24). The live-virus vaccines were produced first, followed by the more sophisticated preparations as methodology improved. In this paper, we discuss HVS vaccines from 3 points of view, which are: (a) inactivation of HVS by heat and formalin, (b) production of temperature-sensitive HVS mutants, and (c) *in vitro* attenuation of HVS.

**Inactivation of HVS by Heat and Formalin.** The original experiments of Ablashi et al. (2) subjected HVS to a temperature of 56° for 0.5 hr. This heat-treated virus still induced malignant tumors in owl monkeys. This work was continued by Laufs (28), who showed that the survival curve of HVS is multicomponental, both after treatment with heat and after treatment with formaldehyde. This situation may thus resemble that of Simian Virus 40 (20). Laufs did, however, develop a killed HVS vaccine by combining heat treatment with formaldehyde treatment (28), and experiments with this type of vaccine are described in this Symposium (29). In view of the results with tumor cell transplantation, this system should provide a means to study the role of tumor cell surface antigens and the expression of the viral genome in oncogenesis. The solution of immunological and biochemical problems of this nature may ultimately be important in the understanding of possible viral etiology of the human tumors.

The long-term effects of inoculation of HVS DNA are not known, and this system should also be very useful in the study of this problem. This has received added importance from the fact that HVS DNA has several components (18),...
one of which is infectious for simian cell cultures, as well as oncogenic in a cottontop marmoset, and another may be able to induce cell transformation in the absence of infectivity (19).

Temperature-sensitive Mutants. Temperature-sensitive mutants of herpesviruses have also evoked considerable interest. These may play an important role in studies dealing with virus-induced cell transformation, especially with Herpes simplex virus (21, 25, 39, 40). The use of these mutants may depend on the fact that they will serve as nonreplicating antigens at the body temperature of the host. Didier et al. (12) propagated HVS at 33° in rhesus monkey (Macaca mulatta) embryo spontaneously transformed cells; this resulted in much lower virus production and virtually eliminated cytopathic effects. The time between HVS inoculation and tumor detection in owl monkeys was prolonged.

Falk et al. (16) performed similar experiments with HVS passaged at elevated temperatures. These experiments are described in this Symposium, and delineate an ideal primate system for the study and comparison of latent versus malignant infection with herpesviruses.

Attenuation of HVS. Passage of viruses in cell culture systems has often resulted in loss or reduction of in vivo infectivity or pathogenicity. Thus, murine Rauscher leukemia virus, which has been passaged in vitro in either mouse (G. R. Armstrong and R. A. Manaker, unpublished observations) or human (5) cells, loses its ability to induce splenomegaly in mice. Similarly, virulent Marek’s disease virus passed in culture became attenuated and ultimately became useful as a live vaccine against Marek’s disease (10). Mouse cytomegalovirus passed in culture loses its ability to cause illness in vivo (13). Meléndez et al. (30) attempted to produce an attenuated HVS strain by passaging the virus in dog embryo lung cells 30 times over a period of 4 years. The virus showed increased replication in dog embryo lung cells and decreased ability to replicate in owl monkey kidney cells, and induced neutralizing antibodies to HVS after inoculation of cottontop marmosets. However, the animals developed tumors after a prolonged incubation period and were not protected from tumor induction by parental HVS (L. V. Meléndez, personal communication).

In another study, Ablashi et al. (1) followed an owl monkey that shed HVS over a period of 1 year but did not develop cancer. However, HVS isolated from this animal was completely neutralized by goat anti-HVS and did induce lymphomas in cottontop marmosets. This finding stresses the observation that one must use the most sensitive test system in demonstrating the potency and safety of a particular preparation; the most sensitive indicators in the case of HVS-induced lymphomas are cottontop marmosets.

The many approaches and findings described and discussed in this paper indicate the complexity of the situation and the need for further research in this area, especially if one possible ultimate result may be the protection of humans against certain forms of cancer. One potentially fruitful line of research with HVS would be the use of subviral components, such as soluble and membrane antigens, in vaccine preparations. These would be free of viral nucleic acid and thus would serve as models for a potential human situation. One approach could be the use of heat- and formalin-treated virus as a source of complement-fixing soluble antigen free of HVS nucleic acid, because the heat-formalin treatment does not completely destroy complement-fixing activity. Membrane preparations from the 39°-passaged HVS could be used in another approach. Other HVS products, such as early and late antigens, could also be purified and used in vaccine studies. Thus, the formulation of a protective vaccine not containing viral nucleic acid would serve as a most desirable model for EBV or Herpes simplex virus type 2 vaccines.

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References


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