# Prevention of Herpes-Associated Malignancies in Primates: Problems and Prospects[1]

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Herpesviruses are known to induce carcinoma, leukemia or malignant lymphoma in different animal species. Herpesviruses are also suspected as being causal agents in human malignant neoplasia. In particular the Epstein-Barr virus (EBV) stands first among candidate human cancer agents (1). The present knowledge indicates an aetiological relationship between EBV and two human malignant tumors: Burkitt's lymphoma and nasopharyngeal carcinoma (2, 3). Herpesviruses are transmitted horizontally and hence are subject to immunologic intervention, specifically by vaccines that stimulate immunity and prevent or limit proliferation of the naturally acquired virus on subsequent infection (4).

The first example of a naturally occurring malignant tumor to be controlled in this way was the herpesvirus induced Marek's lymphoma. A live attenuated herpesvirus almost completely prevents this neoplastic disease of chickens (5). Live virus vaccines in general induce higher level and longer lasting immunity than killed vaccines and require only a small dose of virus to immunize. However, there are no reliable in vitro markers for the oncogenicity of live attenuated herpesviruses that might apply to man. The applicability of live attenuated vaccines derived from potentially oncogenic herpesviruses seems slight.

Therefore killed vaccines derived from the oncogenic herpesviruses have to be developed. At the present level of knowledge only killed cancer virus vaccines seem to be administerable to man. For the control of those human cancers suspected of having a herpesvirus cause a killed vaccine completely free of viral DNA would be desirable, since traces of the viral DNA in such a preparation might be able of bringing about malignant transformation (6). Chickens can be significantly protected against Marek's lymphoma by killed vaccines free of virus nucleic acid (7). A viral nucleic acid-free vaccine for EBV could be prepared by purification of plasma membranes from human lymphoid cells which do have EBV-determined membrane antigens expressed on the cell surface. Antibodies to these antigens also have virus neutralising activity (8).

The preparation of nucleic acid-free herpesvirus vaccines would probably solve the safety problems. But a vaccine has not only to be safe, it also has to be efficient against the natural occuring infection. Even if an EBV vaccine free of nucleic acid is available the efficacy of the killed herpesvirus vaccine in man remains to be determined. Killed whole herpesvirus types 1 and 2 vaccines prepared for preventing or treating primary or recurrent acute episodes of herpesvirus infection in man, have not been highly encouraging in terms of effectiveness. Because of the

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anticipated long incubation period for herpesvirus cancer in man, it may be a long time before the protective efficacy of a killed EBV vaccine can be measured in terms of cancer prevention (4).

Since the oncogenic herpesviruses isolated from nonhuman primates, H.saimiri (HVS) and H.ateles (HVA) regularely induce malignant lymphoma in nonhuman primates within 1–2 months after infection (9, 10) we used these viruses in animals phylogenetically related to man to study the safety and efficacy of killed herpesvirus vaccines. The work with the primate model system offers a wider range of experimental possibilities and the questions in respect to the efficacy of killed vaccines against herpesvirus induced cancer can be answered within a short period of time.

The vaccines were prepared by inactivation of HVS (strain S.295C, friendly supplied by Dr. L. V. Meléndez) and HVA (isolate No. 810, friendly supplied by Dr. L. V. Meléndez) with heat (56 °C for 4 hours) and formaldehyde (100 µg HCHO/ml for 6 days) as recently described (11). The 100-fold concentrates of the virussuspensions which were free of serum and cells, were used as vaccines. The virus specific antigenicity of the vaccines was determined by the complement fixation (CF) test. The DF titers ranged between 1:32 and 1:64. For immunisation four to six intramuscular inoculations of the vaccine adsorbed on to Aluminiumhydroxydgel as adjuvant were given to each monkey within 12 weeks (11).

The killed vaccines proved to be safe in 121 vaccinated monkeys of four different species (S.oedipus, C.jacchus, A.trivirgatus and C.aethiops) (12, 13, 14). Several of these monkeys have now been under observation for two years without any sign of a clinical disease. The vaccines induced high titers of neutralising and complement fixing antibodies in all vaccinated monkeys. Even 9 vaccinations given to a monkey did not induce any kind of incompatibility. The killed herpesvirus vaccines were not only free of infectivity and immunogenic but proved also to be non oncogenic. In spite of the fact that the vaccines are not free of viral nucleic acid, none of the 121 vaccinated monkeys developed a tumor. In contrast to the tumor bearing monkeys and to the latently infected monkeys HVS and HVA could not be isolated from fresh peripheral white blood cells from the vaccinated monkeys. In all our in vivo experiments infectivity and oncogenicity of HVS and HVA are very closely correlated. One single infectious particle was able to induce a tumor and we never found a tumor which did not produce complete virus particles after cocultivation in vitro. The survival curve of HVS in vitro is multicomponential after treatment with heat as well as after treatment with formaldehyde. The inactivation of the oncogenicity followed that of the infectivity.

The vaccinated monkeys (S.oedipus and C.jacchus) were resistant against the intramuscular challenge with 200–300 $LD_{50}$ of cell-free oncogenic herpesvirus (12, 13). The challenged animals remained clinically well without signs of an infection and have now been under observation for 1–2 years while the non-vaccinated control monkeys died of malignant lymphoma 34–52 days after inoculation. The resistance against the oropharyngeal route of infection remains to be determined. This experiment seems of great importance since the natural route of infection is different from that used in the challenge experiments described. Therefore the oropharyngeal route of infection has to be used for future challenge experiments.

In certain virus infections, e.g. measles, infectious hepatitis, German measles,

the passive immunisation by administration of specific serum antibodies during the incubation period may result in prevention or modification of the clinical disease. We investigated if in analogy to these virus infections a state of relative temporary insusceptibility to the oncogenic HVS can be induced in nonhuman primates (S.oedipus) by the administration of antibodies against HVS which have been formed in another host. The passive immunisation with hyperimmune serum against HVS obtained from tumor bearing animals as well as hyperimmune serum obtained from the vaccinated monkeys protected against malignant lymphoma when the monkeys were challenged with 30–40 $LD_{50}$ of cell-free HVS 24 hours after the administration of the specific serum antibodies (15).

The vaccination with the killed oncogenic herpesviruses did not prevent but delayed tumor development after tumor cell transplantation (14). Humoral antibodies do not protect against the cullular transmission of herpesviruses (16). We could demonstrate that the killed HVS vaccine induces a specific cellular immunity in marmoset monkeys (S.oedipus) as well as in C.aethiops monkeys. However, in freshly prepared tumor cells HVS specific antigens could not be demonstrated so far.

The fact that the killed HVS and HVA vaccines were not capable of bringing about malignant transformation in 121 monkeys vaccinated within 1–2 years does not justify the use of an EBV vaccine prepared in the same way in man. A similar prepared EBV vaccine, however, could be used to study the efficacy of a killed EBV vaccine in nonhuman primates. Such studies are now possible since it was shown that EBV induces malignant lymphoma in S.oedipus and A.trivirgatus (17, 18).

We applied the method developed for the production of the killed HVS and HVA vaccines to EBV. For vaccine production the EBV producing human lymphoid cell line P3 HR 1K (1) and the EBV producing marmoset lymphoid cell line B 95-8 (19) were used. The CF titers of the killed vaccines were considerably lower than those obtained with HVS and HVA and ranged between 1:1 and 1:16. The killed EBV vaccines were used for the immunisation of 10 C.jacchus monkeys. The monkeys received 5 inoculations within 10 weeks. As yet all of the animals remained clinically well. The killed vaccines induced specific humoral antibodies against EBV.

We are working at a cell membrane vaccine against EBV which is free of viral DNA and which could be used in man. Preliminary experiments indicate, however, that it is difficult to prepare membrane vaccines which contain enough EBV specific antigenicity to induce a potent immune response. It seems doubtful if such a membrane preparation will ever be economically practical for vaccines. Further the immunologic response can sometimes enhance as well as prevent or suppress tumor. A membrane vaccine enhanced tumor growth in mice induced by the mouse mammary tumor virus (20).

The killed EBV vaccines prepared by the same procedure as the HVS and HVA vaccines as well as DNA free membrane vaccines tested in the nonhuman primate system might be expected to yield a great amount of data on safety, efficacy, and regimen that can be extrapolated to a human vaccine. However, it seems still questionable if a killed EBV vaccine will be efficient against the human tumor suspected of having a herpesvirus cause.

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