Practical Considerations in the Development of a Human Cancer Vaccine

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Summary

Factors involved in the development of a human oncornavirus vaccine are discussed. The isolation and purification of subviral gp69/71 antigenic components enhance the feasibility of developing a safe vaccine. The recent isolation of a C-type virus (the HL-23) from a human leukemic patient and its similarity to the simian sarcoma virus presents us with a unique opportunity to test the safety and potency of a vaccine in nonhuman primates.

Introduction

Several studies have been reported in which laboratory animals being immunized with either formalin-inactivated or live attenuated oncornavirus vaccine are protected against virus-induced neoplasia. With the assumptions that some types of neoplasia are transmitted horizontally by an oncogenic virus, as the case appears to be with feline leukemia (2, 3, 8, 10), then the use of vaccines might be of value in protection against those cancers. However, if the viral genome is transmitted vertically or by prior infection, then the value of vaccine immunization in the suppression of virus genetic expression and control of tumor development is more difficult to predict with optimism.

Discussion

In the development of viral vaccines, we can consider 3 main types: type 1, live attenuated virus; type 2, inactivated whole virus, and type 3, purified subviral components that have been rendered free of viral nucleic acids.

Vaccines representing types 1 and 2 have been described for animal leukemias (4, 5, 11–13, 16). We published our results on successful attenuation of Rauscher virus and the excellent protection it provided to mice when challenged with leukemogenic virus or tumor cells transformed by the virus (13). However, in a later study, we noted inconsistencies with some virus preparations that exhibited significant leukemogenic activity, even after many passages in tissue culture. In addition, we noted that the leukemogenic activity of the virus is dependent upon the type of the host cell used to propagate the virus. Many very difficult problems are associated with the successful and safe use of live oncogenic virus vaccines in human populations (9). The prospect of developing such a live vaccine for human cancer in our opinion is a very remote possibility, but long-term thinking should be given to consideration of that approach, particularly as more information is developed about latent viruses that do not trigger neoplasia but that may engender immunity.

In 1966, we described (12) the preparation of a model formalin-inactivated RLV vaccine following some guidelines established by the Division of Biologic Standards for human formalin-inactivated virus vaccines. Since then considerable progress has been made in several areas concerning safety and potency tests.

The major factors to be considered in the development of inactivated or subviral vaccines are: (a) virus strain; (b) virus propagation on a large scale; (c) feasible means of virus purification; (d) method of virus inactivation; (e) extraction of purified glycoproteins; (f) safety testing; (g) potency testing; (h) stability trials.

Virus Strain. The selection of virus strain for preparation of a cancer vaccine is of paramount importance. Ideally, the virus antigens should be able to provide protection against a variety of neoplasms possibly triggered by related viruses. In reality, however, we now have only a few viruses that can be chosen for experimental purposes, and the properties of these viruses do not necessarily make them suitable candidates for vaccines. The reported (6) isolation of a C-type virus, the HL 23 strain, from a patient with acute myelogenous leukemia has provided an agent that can be considered for the development of an experimental vaccine. Since HL 23 virus has been shown to be related to SSV-1, the utility of such a vaccine can be examined in marmosets or woolly monkeys. Similarly, inactivated SSV-1 or its subviral components (e.g., gp69/71) can be tested in such vaccination programs.

Propagation of Virus on a Large Scale. Primate viruses have been shown to grow in a variety of tissue cultures. At present 100- to 200-liter batches of SSV-1 are prepared in our laboratories from chronically infected human lymphoblastoid NC-37 cells. This virus also multiplies readily in diploid human (WI 38) cells or in primary monkey cells.

Purification of Virus. Concentration and purification of the candidate virus can be carried out by 2 cycles of band-

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2 The abbreviations used are: RLV, Rauscher leukemia virus; SSV (-1), simian sarcoma virus (-1).
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Growth

Plant 100 Pfizer flasks with SSV-1 cells in RPMI 1640 + 8% FCS
Harvest 50 liters after 5 days - store 4°C
Refeed cells with RPMI 1640 + 5% FCS
Harvest 50 liters 2 days later
Pool two harvests (100 liters)

Purification

Clarify in J-21 CF centrifuge
Band in 15-60% isopycnic sucrose gradients in K-II
Remove, pool cuts 25-66% sucrose, dilute
Reband pool in 15-60% isopycnic sucrose in CF-32
Concentration

Remove, pool and dilute virus containing sucrose fractions
Pelletize, resuspend pellet in appropriate diluent

Chart 1. Preparation of purified SSV-1. Diagram showing steps in preparation and purification of SSV. One hundred liters of SSV were harvested from human lymphoblastoid cells (NC-37) chronically infected with the virus. Purification was achieved by clarification in a J-21 CF centrifuge followed by double banding in isopycnic sucrose gradients. Sucrose bands containing the virus were pooled and pelleted by high-speed centrifugation. The pellets were resuspended in an appropriate diluent, frozen, and stored at -70°C. RPMI, Roswell Park Memorial Institute. FCS, fetal calf serum.

ing in sucrose gradient. Chart 1 shows diagramatic scheme of concentration and purification of 1 batch of SSV-1 virus. The virus bands at a buoyant sucrose density of 1.16 g/ml in isopycnic centrifugation. Internal labeling of SSV-1 with tritiated amino acids followed by polyacrylamide gel electrophoretic analysis can detect the major structural proteins of the virus as shown in Chart 2. Of major importance in a vaccination program is the gp69/71 glycoprotein which has been shown to stimulate neutralizing antibodies upon immunization. Upon their isolation, these proteins can be assayed by a radioimmunoassay similar to that shown in Chart 3. Purification of SSV-1 virus by the procedure outlined above results in the following: (a) 2000-fold concentrated virus; (b) virus concentrates that contain between 10^11 and 10^12 virus particles/ml; (c) a total protein content of 1.0 to 1.5 mg/ml; (d) final concentration of fetal calf serum less than 1 ppm.

Method of Virus Inactivation or Extraction of Purified Glycoprotein. Formaldehyde solution still offers the most reliable chemical to inactivate infectivity of the virus by its covalent combination with the amino groups of purines and pyrimidines and thus destroys the ability of the viruses to function as templates or messengers. Formaldehyde treatment was found not to be very mutagenic. Recently, we studied the inactivation kinetics of RLV with a 4,000 dilution of formalin at 4°C. Infectivity endpoints were determined by the XC test system. As can be seen in Chart 4, the virus was inactivated after 72 hr. Virus preparations inactivated in this manner for 7 days followed by neutralization of free formaldehyde with sodium bisulfite provided protection of mice against challenge with leukemogenic virus.

Similar studies are planned to determine the inactivation curve for SSV-1. Infectivity of the virus is assayed by foci formation on normal rat kidney cells (1).

Extraction of Purified Glycoproteins. Purification of gp69/71 (7, 14, 15) glycoprotein has been described for several oncogenic C-type viruses. Basically, purification is accomplished by chromatography of ruptured virus on BioGel, concanavalin A-Sepharose, phosphocellulose, DEAE columns, or a combination of 2 types of columns.
Conclusion

Although the nature of virus involvement in human cancer is still a mystery, we can at this point venture an opinion and prediction. An increasing array of evidence has accumulated in the past 2 years to suggest some correlation between presence of virus genome and the expression of the disease. The isolation of SSV-1-like virus from a patient with myelogenous leukemia points toward that direction, and it creates a sense of urgency to develop preventive approaches to control the disease.

The current reports from several laboratories encourage us to consider the possibility of using specific structural components rather than a whole virion for developing a vaccine. In particular, the immunological properties of purified structural proteins or glycoproteins, free of viral nucleic acid, are of greatest interest. The 2 major structural components that have been recently studied are the glycoprotein of the virus envelope (gp69/71) and the core protein (p30). They have been found to carry multiple antigenic determinants, including those rendering type, group, or interspecies specificities. Group-specific and type-specific determinants of gp69/71 are apparently located on virus envelope and thus are readily accessible to neutralization by specific immune serum. In the mouse model, antiserum to purified gp69/71 of the RLV has been shown to neutralize infectivity, and perhaps the tumorigenic potential as well, of several naturally occurring murine oncogenic viruses. Furthermore, these group-specific determinants have also been found on the surfaces of infected cells, allowing the immune serum to interact and produce lysis of unwanted cells under proper conditions. Therefore, the use of a purified gp69/71 or other protein subunits with group-specific immunogenic determinants seems to be a most realistic approach in developing a vaccine for a human cancer virus.

References

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