Practical Considerations in the Development of a Human Cancer Vaccine

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Summary

Factors involved in the development of a human oncorna-virus 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 oncorna-virus 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 neoplasias 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-
the XC test system. As can be seen in Chart 4, the virus was
function as templates or messengers. Formaldehyde treat-
ment was found not to be very mutagenic. Recently, we
studied the inactivation kinetics of RLV with 1:4000 dilution
of formalin at 4 ~ Infectivity endpoints were determined 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 1011 and
1012 virus particles/ml; (c) a total protein content of 1.0 to 1.5
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 treat-
ment was found not to be very mutagenic. Recently, we
studied the inactivation kinetics of RLV with 1:4000 dilution
of formalin at 4 ~ 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 formal-
dehyde 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.

Chart 1. Preparion of purified SSV-1. Diagram showing steps in prepara-
tion 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 isopyknic sucrose gradients. Sucrose bands containing
the virus were pooled and pelletized by high-speed centrifugation. The
pellets were resuspended in an appropriate diluent, frozen, and stored at
-70 ~ RPMI, Roswell Park Memorial Institute. FCS, fetal calf serum.

Chart 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic analy-
sis of the 3H-labeled proteins of SSV derived from chronically infected NC-37
human cells grown in the presence of a mixture of 3H-labeled amino acids.
Labeled virions banding at an isopyknic density of 1.16 g/ml were disrupted
with sodium dodecyl sulfate and subjected to electrophoresis on 10% poly-
acrylamide gels containing 0.1% sodium dodecyl sulfate. The gels were then
frozen and sliced into 1-mm sections, and the 3H was eluted from them with
organic scintillation fluid containing NCS solubilizer. The molecular weights
of the viral proteins were determined by comparing their electrophoretic
mobility to marker proteins (denoted by appropriate arrows) subjected to
electrophoresis on parallel gels.

Chart 3. Competition radioimmunoassay for SSV p27. The p27 protein
was derived from disrupted virus that had been purified by double banding in
isopyknic sucrose gradient. The disrupted virus was chromatographed on a
BioGel A-5m column (6). Proteins eluting in the 27,000-dalton range were
further purified by cellulose column chromatography (14). The techniques of
iodination and competition radioimmunoassays were carried out according to
the procedure described by Strand and August (14). In this radioimmunoas-
say, however, monovalent rabbit antisera against SSV p27 was used.
**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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