C-type Virus-associated Antigens and Their Relevance to Human Leukemia Control

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Summary

This review describes oncogenic C-type virus and virus-associated antigens as phenotypic expressions of virus activities in malignant cells and cell-surface antigens acquired by malignant transformation. The range of host-immune responses to these antigens in experimental systems is discussed in relation to the immunotherapy and immunoprophylaxis of human cancer patients.

Introduction

There are a number of reasons for attempting to use the information now available on C-type RNA viruses in the control of cancer. (a) The oncogenicity of C-type viruses in vertebrate species is well established. Recently, the continuous production of C-type particles, similar to those isolated from certain primate species, has been reported in cultures of leukocytes from a patient with acute myelogenous leukemia (21). (b) In contrast to chemically induced tumors, cells transformed by a given virus share common antigens that are determined by the viral genome, either as virus structural antigens or virus-associated antigens, or both. (c) Several antigens have been isolated, purified, and characterized with respect to the serological reactivities directed against them. Some of these are expressed at the cell surface whether or not virus particles are produced. (d) Naturally occurring antibodies to C-type virus components and virus-associated antigens have been demonstrated in the animal host. While the correlation of the immune responses with the incidence of cancer should be determined, active or passive immunization may be an approach to the control or prevention of cancers of animals and possibly of man.

In this review, we will attempt to identify C-type virus and virus-associated antigens as phenotypic expressions of virus activities in malignant cells and cell-surface antigens acquired by malignant transformation. Using this information, we will consider the range of host-immune responses that might be evoked by the antigens in experimental systems and indicate directions that may become important for the immunotherapy or immunoprophylaxis of virus-induced tumors.

Analysis of Antigenic Specificity: Virus, Virus-associated Antigens, and Tumor-specific Antigens

C-type virus-induced tumors contain 3 major groups of antigens: virus antigens, virus components themselves; virus-associated antigens, viral genome-coded antigens, excluding virus antigens; and tumor-specific antigens, cellular genome-coded antigens acquired by malignant transformation. The presence of the last group of antigens has not yet been confirmed in mammalian systems. Since RNA tumor viruses can incorporate viral genetic material into the genome of the host cell, varying degrees of expression can occur in infected cells: (a) virus production without transformation; (b) virus production and transformation; and (c) transformation without virus production (nonproductive sarcoma cells in vitro). While maximal virus expression occurs under Condition b, partial expression (production of viral products) occurs in all of the above situations.

Most germane to the purpose of this report are those antigens that are expressed on or are closely associated with the cell surface, because it is at this site that the immunological mechanisms of the host may act to prevent or control infection and/or malignant transformation. Since both virus and virus-associated (non-virus) antigens may occur on the cell surface, the origin of the antigens found there is difficult to distinguish. The complete biochemical characterization of the components in each group may eventually help to resolve this problem.

Viral Antigens. Studies on the isolation, purification, and characterization of mammalian C-type virus antigens have progressed rapidly within the past year. The availability of large quantities of purified viruses, improved isolation procedures, and rapid, sensitive immunoassays have produced many new findings. Several proteins (at least 6 or 7) are present in sufficient quantities in mammalian viruses to be considered major components. With few exceptions, the biological role of these antigens is not well understood (8, 11–13, 53). The proteins possess a spectrum of antigenic determinants with wide ranges of specificity: (a) type-specific, found only in a given virus within a species; (b) subgroup-specific, found in some strains of viruses within a species and different species; (c) group-specific (species-specific), found in all viruses within a species; and (d) interspecies-specific, found in many viruses from different mammalian species. Thus far, mammalian C-type virus antigens have shown no cross-reactivity to avian C-type, mammalian B-type, and Mason-Pfizer monkey viruses (53). The current knowledge about these antigens, based primarily

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2 Presenter.
the most striking feature of virus-associated CSA is that the tumors induced by a given virus, even in different species, share a common CSA (44, 45). Different viruses induce CSA with different specificities. This feature of common CSA specificity is of great advantage to immunotherapy of virus-induced tumors. On the other hand, it is a disadvantage that virus-infected somatic cells also acquire CSA common to the CSA of malignant cells, regardless of virus production (see below). Solubilized MuLV-associated surface antigens were fractionated into 2 peaks of 60,000 and 45,000 daltons (25). However, the solubilized surface antigens are labile, being inactivated by 2 cycles of freeze-thawing or storage at 4°C for 1 week.

Besides virus-induced CSA, a recent study has shown that CSA determinants common to the specificity of VEA are present and consist of gp70 (35). The antiserum against gp70 isolated from the surface of NZB nonproductive lymphoma cells neutralized C-type viruses released from NZB productive lymphoma cells. The presence of p30 on the cell surface has also been demonstrated (67). In any discussion of CSA, the following points should be clarified: (a) Is a certain CSA a component of the plasma membrane? For example, the presence of CSA common to VEA was demonstrated on the malignant cells in the mouse system and nonmalignant cells in the avian system (23, 35). This antigen seems most likely to be a constituent of the plasma membrane, because nonproductive cells contain this antigen. Furthermore, G17 antigen in the Gross (G) leukemia system has been considered to be induced by interaction between cellular and viral genomes (55), and purified as gp70 (not-related VEA) from normal thymocytes of mouse strain 129 (19, 42, 64). (b) Are other surface antigens common to virus antigens adsorbed on the plasma membrane? It has been well known that circulating soluble antigens including intraviral components are adsorbed onto the cell surface in vivo and in vitro (4, 59), a kind of antigenic conversion (60). Additionally, intraviral components were also proven to exist in the circulation, since these antigen-antibody complexes were found in the mouse kidney (28, 47). Therefore, p30 seems most likely to be the intraviral component derived from disrupted viruses or cells and attached on the cell surface.

**Tumor-specific Antigens.** Analogous to the results of individual antigen specificities of mouse mammary tumor virus-induced mammary tumors (40), it is possible that C-type virus-induced tumors carry individual antigen specificities acquired by malignant transformation. Preliminary results indicate that some AKR spontaneous leukemias share a common CSA but others contain individual specificities (E. Okazaki, unpublished data). This CSA may be very important as an approach to tumor immunotherapy. In particular, the presence of common CSA of this category suggests that C-type virus selectively infects the special part of the cellular genome. These findings on CSA are summarized in Table 2.
Naturally Occurring Antibodies

In mouse systems there are many reports on the occurrence of antibodies to internal and envelope components of C-type viruses. Because of antigen excess, the antibodies were found mainly in the form of immune complexes in the glomeruli of the kidney (28, 47, 68). More recent studies show that mouse strains with low incidences of spontaneous lymphoma produce antibodies to MuLV's. In particular, free antibodies in the sera of these mice recognize proteins of the viral envelope with approximate molecular weights of 68,000 (gp70), 43,000 (gp43), and 17,000 (p15) daltons (9, 24, 29, 31, 38, 39). Such sera possess weak virus-neutralizing activity. In another study, high tilters of neutralizing antibodies to a C-type virus (BALB: virus 2) were detected in many strains of mice and F, hybrids (1).

In the cat, infection with FeLV appears to be fairly common and neutralizing antibodies to gp70 can be detected in the sera (33). A similar result has been reported for infections by MuLV and gibbon leukemia virus systems. The findings correlate well with the proposed horizontal spread of FeLV, MuLV, and gibbon leukemia virus. Cats, gibbons, and mice also produce antibodies to their homologous p30 antigens (R. V. Gilden, personal communication: Refs. 2 and 34).

There have been several reports that antibodies to interspecies determinants of p30 occur in the sera or renal eluates of cancer patients. If confirmed, this finding would not only constitute presumptive evidence that a C-type virus is associated with human tumors but would indicate that host immune recognition of a tumor antigen occurs. However, other studies conducted using electrophoresed mouse p30 in a sensitive radioimmunoassay failed to detect any antibody activity to this antigen. Adult and juvenile cancer patients and their respective controls, as well as laboratory personnel working with RNA tumor viruses, constituted the test group (17).

Thus, despite the persistence of these viruses throughout their lifetimes, most animals seem to respond to C-type virus antigens. As yet there is little information to determine whether these antibodies benefit the host, i.e., control virus expression or oncogenesis.

When C-type virus-associated surface antigens are considered for use in immunotherapy, special attention should be paid to the antigenic modulation observed on TL antigen (15), GCSA (5), and fetal antigen (49). Some differences can be found in the mode of antigenic modulation of these antigens (Table 3).

The cells of leukemias can be utilized for immunization, since the antigenic modulation of these cells is prevented by formalin fixation (36). On the other hand, it is almost impossible at present to prevent antigenic modulation of malignant cells growing progressively in the host by inoculation of humoral antibody. It still remains to be determined whether cell-mediated immunity induces antigenic modulation. Increased understanding and control of the process of antigenic modulation are necessary conditions for successful tumor immunotherapy using adoptive immunity.

The natural appearance of lymphocytes sensitized to CSA has also been demonstrated (41), but it is still unclear whether the tolerance in terms of cell-mediated immunity to the virus-associated antigens is incomplete or complete (3, 18). Humoral and cell-mediated antibody production appear to show some dissociation; namely, humoral antibody tolerance is incomplete but cell-mediated tolerance may be complete or incomplete (18). This phenomenon has been designated as partial tolerance.

Stimulation of Host Immune Response

As in other virus infectious diseases, tumors in patients can be prevented or treated by immunological methods in a similar way, e.g., passive or active immunization. Additionally, specific as well as nonspecific immunological stimulation can be applied to tumor patients. To date, transplanted tumors have been used in experimental systems by a majority of investigators. However, either spontaneous or primary tumors are preferable as a model system, because once transplanted the transplantation immunity interferes with the natural characteristics of tumor growth. In other words, once primary tumors are removed from the original hosts and transplanted into other sites of the original hosts or into other syngeneic hosts, the tumors behave like foreign bodies.

Specific Immunization

Using tumor-specific antigens, antisera to them can be prepared in other hosts, regardless of the species. These antisera may be utilized for passive immunization with antisera. Antibodies to gp70 show strong neutralizing activity that appears to reside on the protein moiety of this mole-
cule. While antibodies directed to group determinants show the strongest reactivities, interspecies determinants are also involved. This information can be applied to immunoprophylaxis by vaccination with these antigens, the determination of viral etiology, and epidemiology in C-type virus-induced tumors. There are many reports in animal systems that vaccination with formalin-killed virus induces the production of antibodies (including neutralizing antibodies) (20). In some cases, the immunization delayed or prevented the onset of virus-induced oncogenesis, even in the host of origin. The human cancer patient also appears capable of mounting an immune response to formalin-killed Rauscher murine leukemia virus (26). Although the sera did not neutralize virus activity, antibodies to p30 were clearly detectable by radioimmunoassay. This response is augmented when BCG is used as an adjuvant and appears to correlate well with other in vivo and in vitro tests for cellular hypersensitivity. If, in the future, some form of virus vaccine therapy proves effective in controlling pathogenesis in animals, possible application to human disease, i.e., immediately after a remission occurs, may be feasible. Furthermore, by taking advantage of the interspecies cross-reactions among the animal viruses, the dangers of using a human virus isolate may be avoided.

When normal lymphocytes are inoculated into tumor-bearing hosts either after in vitro sensitization or without sensitization, the lymphocytes are expected to be sensitized in vivo (transfer of adoptive immunity). In the syngeneic rat Gross MuLV-induced leukemia system, lysis of target malignant cells occurred in vitro when normal lymphocytes, antisem, and leukemia cells used as target cells were incubated together (50). This finding opens a promising way to adoptive immunotheapy of tumors. Of course, active immunization with tumor-specific antigens can be considered, but the immune response is usually suppressed in tumor-bearing hosts. Thus, active immunization of such hosts frequently does not work well. To overcome this problem, combined therapy with other host immune stimulants should be considered as described below. It has been reported in experimental systems, however, that the active immunization of spontaneous leukemia-bearing as well as preleukemic AKR mice with neuraminidase-treated AKR leukemia cells showed some inhibitory effects on either tumor growth or incidence (27). In addition, when pregnant cats were immunized with FeLV, their progeny showed little or no incidence of leukemia by passive immunization with the mother’s antibody (65). Nevertheless, specific immunization with tumor antigens or virus antigens needs to be studied further.

### Nonspecific Stimulation

Polysaccharides, lipopolysaccharides, BCG, and other microorganisms have been used as nonspecific stimulants to enhance tumor patients’ immune response. These materials sometimes share an antigen common to antigen(s) on the surface of some tumors (16).

Here, we wish to describe a preparation of dried Streptococcus hemolyticus with low virulence (OK-432) which has 2 effects on malignant cells: (a) the direct effect of inhibiting synthesis of new cell DNA, RNA, and proteins (48); and (b) the indirect effect of enhancing host immune response (43). We reported that coccus-treated C57BL/6 and BALB/c mice produced natural antibodies to GCSA and PC1 antigen, respectively, at higher titers than those produced by single immunization with these specific antigens, and that these natural antibodies did not react with the coccus preparation (7). In another experiment, when OK-432 was continuously injected i.m. twice a week into preleukemic mice, 55% of the OK-432-treated mice developed spontaneous leukemias in contrast to a 95% leukemia in 0.9% NaCl solution injection-treated or nontreated mice (controls) at the age of 11 months (Aoki et al., unpublished observations). To distinguish whether OK-432 inhibited virus replication or malignant cell division, the reverse transcriptase activity in the plasma and interferon in the serum were assayed in both groups at the preleukemic stage after OK-432 treatment. The OK-432-treated group was negative for reverse transcriptase activity and positive for interferon, whereas the reverse was found in the control group. Thus, it seems most
likely that OK-432 treatment inhibited virus replication at the viral genome level, since the direct effect of cocci on malignant cells may release reverse transcriptase through the damage to these cells. It cannot be ruled out, however, that OK-432 may inhibit malignant cell division at an undetected level.

An ideal antitumor agent consists of one that has an inhibitory effect on malignant cells and that at least does not suppress or, if possible, enhances the host immune response to surface antigens of tumors. To a certain extent OK-432 appears to satisfy these requirements of an antitumor substance.

Application to Human Tumors

The presence of C-type viruses in human tumors remains to be proven definitively. Suggestive reports in the literature, however, warrant consideration of the immunotherapy and/or immunoprophylaxis of tumor patients in terms of C-type oncogenic virus. In applying experimental results to human immunotherapy and immunoprophylaxis, the following precautions should be considered: (a) prevent damage of virus-infected somatic cells; (b) avoid the risk of inducing glomerulonephritis through deposits of antigen-antibody complexes in the kidneys by any means; and (c) satisfy ethical demands by avoiding biohazards. Since C-type virus-infected, nontransformed cells share common CSA with C-type virus-induced malignant cells, both somatic and malignant cells may be damaged when either humoral or cell-mediated antibody to the CSA attacks these cells. To avoid this risk, one might take advantage of the different sensitivities to antibodies between somatic and malignant cells; thus, somatic cells may be more resistant than malignant cells to antibodies. For example, in the Gross leukemia system, malignant cells were destroyed by direct cytotoxic tests but the presence of GCSA of somatic cells could be proven only by absorption tests (46). In another way, the tumor-specific CSA acquired by malignant transformation could be separated from virus-associated CSA, and the purified antibody to tumor-specific CSA would be applied to the selective cytolysis of malignant cells. However, this problem also remains open.

Will the malignant cells used for immunization contain C-type virus and/or viral genome that may induce new tumors in the host? Formalinization of malignant cells before use in immunization and the use of purified antigens constitute 2 ways of preventing the introduction of oncogenic materials. Even in experimental models, ethical consideration must be made in studies of tumor immunotherapy.

BCG treatment and transfer of normal thoracic duct lymphocytes have been used for immunotherapy of tumor patients; BCG treatment showed marked effects on skin tumors, mainly melanomas (14), and thoracic duct lymphocytes significantly improved the condition of 1 of 4 leukemic patients (66). One of the authors treated patients with advanced gastric carcinomas with zymosan (yeast polysaccharides), and the patients gained temporal remission (6). A preparation of *Streptococcus hemolyticus* (OK-432) has been used for treating patients with various tumors, and quite promising results have been obtained in certain tumors including leukemias (37). In terms of specific immunotherapy, several approaches can be considered (Chart 1). To warrant the application of these approaches to specific immunotherapy of human tumors, each step should be examined, e.g., how to prepare safe tumor-specific antigens, how to produce antisera to these antigens, how to avoid biohazard risks that may be caused by immunotherapy, etc.

Finally, it should be realized that conventional therapy, e.g., irradiation, chemotherapy, surgery, etc., remain the mainstay of cancer therapy and that immunotherapy is currently being considered as an adjunct. Optimal therapeutic effect will be obtained by the proper combination of methods, including immunotherapy. Operable tumor patients can be treated by combining surgery and any other therapy. On the other hand, use of appropriate combinations of methods other than surgery need to be considered for inoperable patients. In this case, we must at least refrain from using methods that suppress the host immune response. If possible, immunotherapy should be combined with chemotherapy that increases host immune response.
References


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