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Immunogenic Properties of a Nonproducer Malignant Tumor Induced by Murine Sarcoma Virus (39445)

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Murine sarcoma virus (Moloney) M-MSV(M-MuLV) and other pseudotypes of murine sarcoma virus induce tumors in intact adult mice that invariably regress. These tumors have been inappropriately designated rhabdomyosarcomas or sarcomas (1). The major portion of the tumorous mass arising following intramuscular or subcutaneous injection of virus in mice is a reactive mass ("atypical granuloma") which does indeed contain varying proportions of malignant mesenchymal foci (2). Through passage of some of these tumors in immunologically deficient mice it has been possible to select malignant populations of cells that will grow progressively and kill intact syngeneic recipients. We have reported the selection and development of hemangiosarcomas by serial passage in thymectomized C57BL/Ka × C3Hf/LW F_1 ($BC3HF_1$) mice of tumors induced originally by M-MSV(M-MuLV) (3). The hemangiosarcoma designated *XM-1* (4) did not produce infectious MSV or murine leukemia virus (MuLV), was negative for *gs* antigen, but contained a defective MSV genome rescuable by Rauscher-MuLV (R-MuLV) and also contained occasional C-type particles. Thus, this parental cell line resembled the 3T3 (S+L−) cell lines induced *in vitro* by M-MSV (5). *XM-1* was found to contain virus-specified transplantation rejection antigens (TSTA) (4).

Clonal derivatives were obtained *in vitro* from single cell isolates of neoplasm *XM-1* at transplant generation 47 (6). Several clones were characterized as to their virus production, presence of *gs* antigen, growth characteristics, and immunogenicity and immunosensitivity in tumor rejection. In contrast to the parental *in vivo* passaged *XM-1* neoplasm, none of the clones contained rescuable MSV. Low levels of infectious MuLV were detected in some clones, but the presence or absence of MuLV did not influence immunogenicity or immunosensitivity of these cloned tumors as detected by virus-specific tumor rejection (6).

One of these clonally derived cell lines, *H-11* (HP) was of particular interest. Infectious virus was not detectable, nor was virus inducible with BrdU. Preliminary data suggested that *H-11* (HP) was highly immunogenic in *in vivo* tumor rejection experiments and tumor growth was inhibited in M-MSV-immunized syngeneic $BC3HF_1$ recipients. We now report further experiments that we believe distinguish between virus-specific, new cell surface antigens (TSTA) and virion antigens in this nonproducer cell line.

Materials and methods. H-11 (HP) cells were grown continuously in Eagle's minimal essential medium with 10% fetal bovine serum (GIBCO) and antibiotics (100 U of penicillin and 100 μg of streptomycin/ml). *H-11* (HP) cells were periodically assayed in syngeneic mice for their tumor-producing capacity. After several passages, progressively growing tumors were produced only with large inocula in the range of 5–10 × 10^6 cells/mouse. Several *in vitro* passages of *H-11* (HP) examined have been negative for infectious virus by XC and MSV focus forming assay. By [^{3}H]uridine labeling and sucrose gradient centrifugation techniques there was no evidence of virus particles with a density of 1.16 g/cm^3 detected in the supernatant, nor was virus associated with RNA-dependent DNA polymerase detected. MSV was not recoverable by cocultivation with MuLV-carrier fibroblasts or superinfection with high-titered M-MuLV or R-MuLV. Neither bromo- nor iododeoxyuridine was capable of inducing infectious virus. MuLV group specific antigens (*gs*) were also not detected in any of the several assays (CF and immunofluorescence). Radioimmunoassay indicates the presence of

gp 70 (13 to 30 ng/10^6 cells). The significance of gp 70 in tumor rejection, however, has not been adequately assessed. It would appear therefore that these nonproducer *H-11* (HP) cells contain, in all likelihood, a strikingly repressed, integrated MSV or MuLV genome. Loss of a potentially integrated viral genome does not appear likely.

Syngeneic (BC3H)F_1 mice were immunized using several regimens of subcutaneously inoculated *H-11* (HP) cells that were trypsinized, washed, and resuspended in serum-free medium. Specificity of tumor rejection was assayed through immunizations with M-MSV(M-MuLV) and with the polyoma virus-induced sarcoma #89 adapted to *in vitro* growth. Because of the low oncogenic potential of the continuously passaged *H-11* (HP) clonal line, tumor challenge was made with *H-11* passaged *in vivo* and then grown in tissue culture (*H-11* short passage). These cells were then aliquoted and frozen. The TD_{50} of this standardized preparation was 1×10^4 cells.

Results and discussion. It may be seen from the results of four separate experiments (Figs. 1A and B and Tables I and II) that immunization with nonproducer *H-11* (HP) cells provided protection in syngeneic hosts against grafted *H-11* tumor cells. This protection was evident in the frequency of progressively growing tumors as well as in

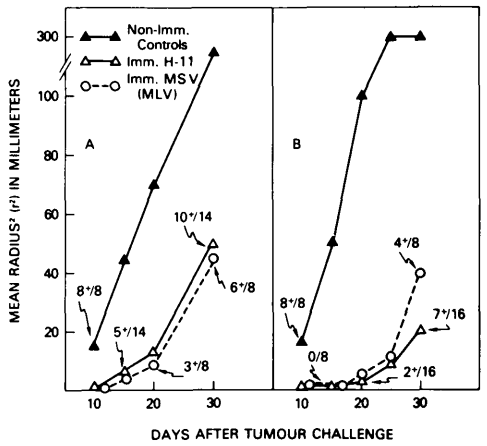

FIG. 1. Syngeneic (BL × C3H)F_1 female mice, 12 to 16 weeks of age, in both groups A and B, were immunized with 2×10^6 nonproducer *H-11* (HP) cells subcutaneously, once a week ×5, or with M-MSV, 1 ml subcutaneously, once a week ×3. Virus concentration = 5×10^5 focus-forming units (ffu)/ml. All groups were challenged 10 days after last immunization with 1×10^5 *H-11* cells. All mice in the A group received 300 R whole body X irradiation 24 hr prior to tumor challenge. (▲——▲), nontreated controls; (△——△), immunized with *H-11* (HP) cells; (○- - -○), immunized with M-MSV. Numbers 8+/8; 10+/14, etc. = fraction of mice with progressively growing tumors. Days of observation = 70; frequencies of tumors did not change beyond 30 days. Mean age to death of controls in group B = 32.6 days; mean age to death of *H-11* immunized mice = 70.1 days. *H-11* (HP) immunized mice also challenged with tumor #89 a polyoma virus-induced sarcoma. See text.

TABLE I. IMMUNOLOGIC SPECIFICITY OF IMMUNIZATION WITH NON-PRODUCER *H-11* (HP) CELLS AND POLYOMA VIRUS.

	Immunization[a]		
Challenge tumor	*H-11* (HP)	Polyoma virus or #89 cells (fraction of mice with progressive tumors at 70 days)	None
H-11 (5×10^4 cells)	1/6[b] (17%)	12/17[c] (70%)	10/12 (83%)
#89 (1.5×10^5 cells)	5/6 (83%)	4/17[d] (24%)	10/12 (83%)

[a] (BC3H)F_1 female mice, 12–16 weeks of age, immunized as follows: *H-11* (HP) cells at $1\text{–}2 \times 10^6$ cells once a week ×5; polyoma virus (LID-1 strain) inoculated weekly ×3 at a concentration of 5×10^6 plaque-forming units per mouse; tumor #89 cells inoculated into footpad and amputation done at 2 weeks. Tumor challenges were done at 2 weeks after the last immunization.

[b] Difference in frequency = $0.5 > p > 0.01$ as compared with the combined data obtained with the other (polyoma immunized and nonimmunized control).

[c] Inhibition of tumor growth also reflected in rate of growth of *H-11* tumor; thus, mean age to death of controls = 40 days and of *H-11* immunized mice = 75 days.

[d] $p < 0.01$ as compared with the combined data obtained with the other two groups (H-11(HP) immunized and nonimmunized control).

TABLE II. IMMUNOLOGIC SPECIFICITY OF IMMUNIZATION WITH NONPRODUCER *H-11* (HP) CELLS.

Challenge tumor		Fraction of mice with progressive tumors (mean radius[2]) at day					Mean age to death (days)
		15	20	25	35	50	
H-11 (10^5 cells)	Immunized[a]	0/8	2/8(2.3)	6/8(9.5)	6/8(50)	–	46
	Nonimmunized	6/8(20)	7/8(64.5)	7/8(>100)	7/8(>100)	–	35
#89 (10^5 cells)	Immunized[a]	2/8	2/8	2/8	7/8	8/8	70
	Nonimmunized	2/8	2/8	2/8	5/8	7/8	70

[a] (BC3H)F_1 female mice, 12–16 weeks old, immunized with *H-11* (HP) tissue culture-passaged cells at 2 × 10^6/week × 5; challenged with tumors *H-11* and #89 10 days after last immunization.

the rate of growth, measured as mean radius[2] or mean days to death of tumorous mice. Preimmunization with the homologous virus strain M-MSV(M-MuLV) also induced resistance to challenge with *H-11*. These results along with those reported earlier of group cross-reactivity among several clones cultured from *XM-1* tumor clearly indicate the existence on *H-11* (HP) cells of virus-specific transplantation antigens. Irradiation (300 R, whole body) of *H-11* (HP) immunized mice before challenge did not abolish their capacity to reject *H-11* syngeneic tumor cells (see Fig. 1A).

The specific nature of the immune response induced by nonproducer *H-11* (HP) cells is seen in the results detailed in Tables I and II. The polyoma virus-induced sarcoma #89 that has its own virus-specific TSTA (7) grew progressively in *H-11* (HP) immunized syngeneic mice but was rejected in mice immunized with polyoma virus or #89 cells. Further, immunization with polyoma virus or #89 cells did not induce rejection of *H-11*.

It is clear, therefore, from these data that MSV-transformed cells (in this study using a clonal line of malignant hemangiosarcoma transformed *in vivo*) do contain MSV-specific surface antigens capable of evoking transplantation immunity. Since no evidence of virus production or of viral fingerprints was found by any of the available assays in our cloned *H-11* (HP) tumor line, virion antigens or surface antigens associated with virus production are not responsible for tumor rejection. MSV-induced new surface antigens, analogous to the TSTA of DNA virus-induced neoplasms, appear to be responsible.

A Harvey-MSV-induced neoplasm of the inbred PD_4 hamster and an M-MSV(M-MuLV)-induced neoplasm of the rat both reported to be "nonproducers" were shown previously (8, 9) to possess tumor-associated antigens of the transplantation type; in both cases, however, MSV could be rescued by "helper" viruses and both contained *gs* antigen. An MSV-associated antigen on the surface of MSV-transformed nonproducer cells was demonstrated by immunoelectron microscopy (10). However, its relationship with TSTA is not known. Others have found no evidence for the existence of distinctive surface antigens on nonproducer MSV-induced neoplasms using serologic (11, 12) or transplantation rejection (13) assays.

Although a complete MSV genome has not been detected, it cannot be ruled out that a protein of the viral genome exists in *H-11* (HP) cells that codes for the TSTA. Experiments are in progress to detect a portion of the virally related genome by molecular hybridization.

Summary. A cloned cell line *H-11* (HP) derived from an MSV-induced neoplasm (a hemangiosarcoma) was found to possess virus-specific tumor rejection antigen(s). The specific nature of the immune response was established through the use of a polyoma virus-induced neoplasm #89 both in cross immunization and cross challenge experiments. Virus antigens or surface antigens associated with virus production are not responsible for tumor rejection since virus production could not be detected, nor were any viral fingerprints of MSV found by the several assays used.

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