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PREVENTION OF SYNGENEIC TUMOR GROWTH IN VACCINIA VIRUS-PRIMED MICE BY IMMUNIZATION WITH VACCINIA VIRUS-MODULATED TUMOR CELLS

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SUMMARY Immunization with vaccinia virus-infected and then X-ray-irradiated murine hepatoma MH134 cells provided C3H/He mice with strong resistance to challenge with viable MH134 cells. Male C3H/He mice of 5 to 6 weeks old were primed intraperitoneally (IP) with 1×10^7 PFU of live vaccinia virus (Ikeda strain) after irradiation with 250 R of X-ray. Three weeks after priming, the mice were immunized IP 3 times at weekly intervals with 1×10^7 X-ray-irradiated MH134 cells that had been infected with vaccinia virus 8 h before irradiation. Over 60% of these cells showed vaccinia virus-induced antigen on their surface (membrane antigen). Challenge with viable MH134 cells was done by inoculating 1×10^5 cells IP one week after immunization. During a 4-week observation period, all the untreated control mice died with ascites. On the contrary, all the mice that were X-ray-irradiated, primed and immunized survived challenge with the tumor cells for at least 4 weeks. The mortalities of mice in other groups that were not irradiated, or not primed, or immunized with only X-ray-irradiated tumor cells, were at lowest 50%.

INTRODUCTION

Tumor cells have been demonstrated to have tumor-associated transplantation antigens (TATA) on their surface (Klein, 1966), and it has been shown that TATA induces immune responses (Herberman, 1974). Many experimental and therapeutic studies have shown potentiation of the immune system against tumors. However, rejection or regression of syngeneic tumors is rare, probably because immunogenicity of syngeneic tumors is generally weak. Hamaoka et al. (1979) reported inducion of cytotoxic killer T

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lymphocytes against a syngeneic tumor in mice by priming with a hapten and then im-imunization with hapten-conjugated tumor cells.

We demonstrated that a virus-induced antigen was expressed on the surface of cells infected with vaccinia virus (Miyamoto and Kato, 1968; 1971). The antigen can induce cell-mediated immunity in rabbits and mice (Ueda and Tagaya, 1973; Oie and Ichihashi, 1981). Consequently, we tried to modulate the tumor cell surface by infection with vaccinia virus to enhance the immunogenicity of TATA in a syngeneic system. We report here that vaccinia virus-primed C3H/He mice become resistant to challenge with viable murine hepatoma MH134 cells when they are immunized with vaccinia-virus infected MH134 cells.

MATERIALS AND METHODS

1. *Mice*

Male C3H/He mice were purchased from the Shizuoka Agricultural Cooperative Association for Laboratory Animals (Shizuoka, Japan) and used at 5–6 weeks old. For passage of tumor cells, we used C3H/He mice of both sexes, which were kindly supplied by Dr. S. Tanabe (Dept. of Bacteriology, Osaka University School of Medicine) and were bred in our animal facilities.

2. *Tumor cells*

CCl_4-induced murine hepatoma MH134 of C3H mice was kindly supplied by Dr. S. Tanabe and passaged serially in C3H/He mice intraperitoneally.

3. *Vaccinia virus*

The Ikeda strain of vaccinia virus, formerly used as a seed virus of smallpox vaccine in Japan, was grown in rabbit kidney-derived RK13 cells (for preliminary experiments) and in chorioallantoic membrane (CAM) of specific pathogen free 11-day-old chick embryos (for main experiment). The virus was harvested from RK13 cells by freezing and thawing the infected cells. The virus was harvested from CAM by homogenizing infected CAM in a whirling blender. In both cases, the virus was clarified by centrifugation at 3,000 rev/min for 15 min at 4 C, and the supernatant fluid was stocked at −80 C. The infectious titer of stocked virus was usually 1×10^9 plaque forming units (PFU)/ml.

4. *Infection of tumor cells with vaccinia virus*

Tumor cells were collected from ascites of C3H/He mice and washed once with Eagle's minimum essential medium (MEM) by centrifugation at 1,000 rev/min for 10 min. Samples of 1×10^8 cells were mixed with 1×10^9 PFU of vaccinia virus in 5 ml of MEM in a 20-ml vial. Then they were incubated at 37 C for 2 h with gentle shaking at 15 min intervals. After incubation, the suspension was mixed with 5 ml of fresh MEM supplemented with 5% calf serum (CS), and incubated further for 6 h at 37 C with gentle shaking at intervals. Then, the cells were collected by centrifugation, and adjusted to 2×10^7 cells/ml with MEM.

5. *Priming and Immunization*

Mice were injected intraperitoneally with 0.5 ml of 2×10^7 PFU/ml of vaccinia virus suspended in MEM 2 or 3 weeks before immunization (priming). After completion of priming, mice received 1×10^7 X-ray-irradiated (5,000 R) MH134 cells, which had been infected with vaccinia virus and suspended in MEM, once a week for 3 weeks. Sixty percent or more of the vaccinia virus-infected MH134 cells showed vaccinia virus-induced cell surface antigen (MA) 8 h after infection.

6. *Fluorescent antibody technique*

MA on the surface of vaccinia virus-infected cells was detected by an indirect fluorescent antibody technique with hyperimmue rabbit serum against vaccinia virus (Ikeda strain) and fluorescein isothiocyanate-conjugated goat IgG against rabbit IgG (Miles Lab, Indiana, USA).

7. *Challenge with MH134 ascites tumor cells*

All mice were challenged intraperitoneally with 1×10^5 viable MH134 cells and observed for 4 weeks. After this challenge, untreated control mice died within 3 weeks with ascites due to increase in MH134 cells.

RESULTS

1. *Kinetics of vaccinia virus-induced cell surface antigen*

A sample of 1×10^8 MH134 cells was in-

fected with vaccinia virus at multiplicities of infection (moi) of 10 and 1 in 5 ml of MEM. Samples of the cells were examined for vaccinia virus-induced cell surface antigen (membrane antigen, MA) at 2 h intervals after infection. As shown in Fig. 1, MA was detected by the indirect fluorescent antibody technique at a maximal level of 60% or more

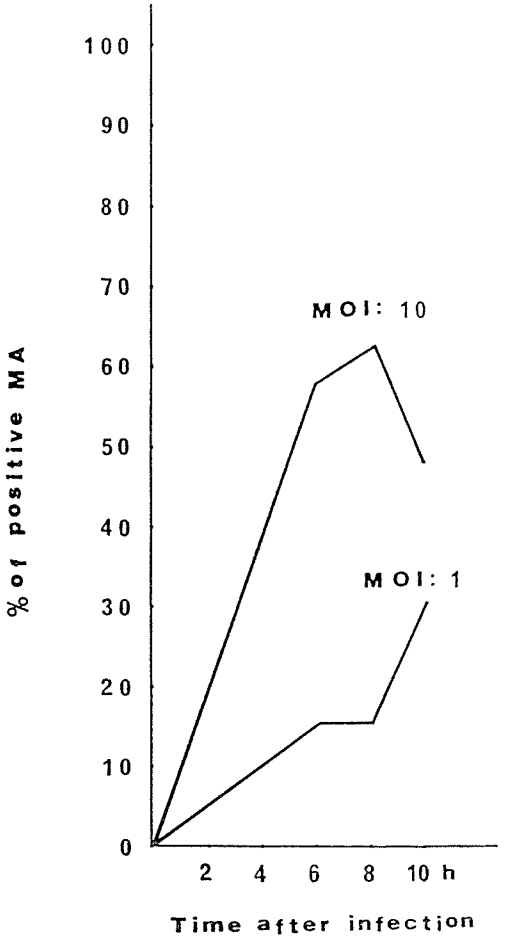

FIGURE 1. Kinetics of change in a vaccinia virus-induced cell surface antigen (MA) on MH134 cells. MH134 cells were infected in vitro with the Ikeda strain of vaccinia virus at multiplicities of infection (moi) of 10 and 1. MA was detected by an indirect fluorescent antibody technique using anti-vaccinia virus (Ikeda strain) rabbit serum and fluorescein isothiocyanate conjugated anti-rabbit IgG goat IgG.

8 h after infection at moi 10. So, we used MH134 cells at 8 h after infection as immunogen (or vaccine).

2. *Preliminary experiments on prevention of death with MH134 cells*

On the day of irradiation with 250 R of X-ray, mice received 0.5 ml of vaccinia virus (1×10^7 PFU) in MEM intraperitoneally (IP) (priming). Two weeks after priming, mice received 1×10^7 MH134 cells, infected with vaccinia virus and irradiated with 5000 R of X-ray and suspended in 0.5 ml of MEM, 3 times at weekly intervals (immunization). One week after immunization, mice were challenged IP with 1×10^5 viable MH134 cells and were observed for 4 weeks.

As shown in Table 1, the 3 untreated control mice died within 3 weeks with ascites, whereas the 8 mice irradiated with 250 R of X-ray, primed with vaccinia virus and immunized with MH134 cells infected with vaccinia virus and then irradiated with 5000 R of X-ray, survived challenge with 1×10^5 viable MH134 cells without showing any evidence of ascites. Omission of X-ray irradiation increased the mortality to 38%. Without either X-ray irradiation or priming, no mice survived challenge with MH134 cells even when they had been immunized with infected and irradiated MH134 cells.

TABLE 1. *Mortalities of mice challenged with MH134 cells*

	Priming	(+)		(−)
	X-ray irradiation	(+)	(−)	(−)
Immunization	none	ND	ND[b]	3/3 (100[a])
	X-ray-MH134 (Vacc.)	0/8 (0)	3/8 (38)	8/8 (100)
	X-ray-MH134	ND	ND	2/9 (22)

[a] Values in parentheses are percent mortalities.
[b] ND: not done.

On the contrary, 7 of 9 mice that had not been irradiated nor primed but had been immunized with uninfected and X-ray-irradiated MH134 cells, survived challenge with MH134 cells.

These preliminary experiments suggest that though MH134 cells alone induce a rather strong immune response in syngeneic mice, vaccinia virus-infected MH134 cells induce a much stronger immune response against tumor associated transplantation antigen (TATA). So, we examined these phenomena in detail.

3. *Effect of vaccinia virus in enhancement of TATA*

Twelve groups of 10 mice were set up differing in whether they received X-ray irradiation, priming, immunization with vaccinia virus-infected and then X-ray-irradiated MH134 cells, X-ray-irradiated but not infected MH134 cells or live vaccinia virus alone, or were not immunized. In this experiment, we extended the period between priming and immunization from 2 weeks to 3 weeks.

As shown in Fig. 2, only the mice that were irradiated with 250 R of X-ray, primed with vaccinia virus and immunized with vaccinia virus-infected and then X-ray-irradiated MH134 cells completely survived challenge with 1×10^5 viable MH134 cells. These mice did not show any evidence of ascites and remained healthy for at least 6 weeks, the time when the experiment was terminated. The mortality of mice immunized with infected and irradiated MH134 cells was increased to 50% when the mice were primed but not irradiated, and to 80% when the mice were irradiated but not primed. Other groups showed even higher mortalities. Among groups immunized only with X-ray-irradiated but not infected MH134 cells, the mortality of the group that had not been irradiated and had not been primed was the lowest, being 60%. In this case, X-ray-irradiation and priming had adverse effects on the mortality.

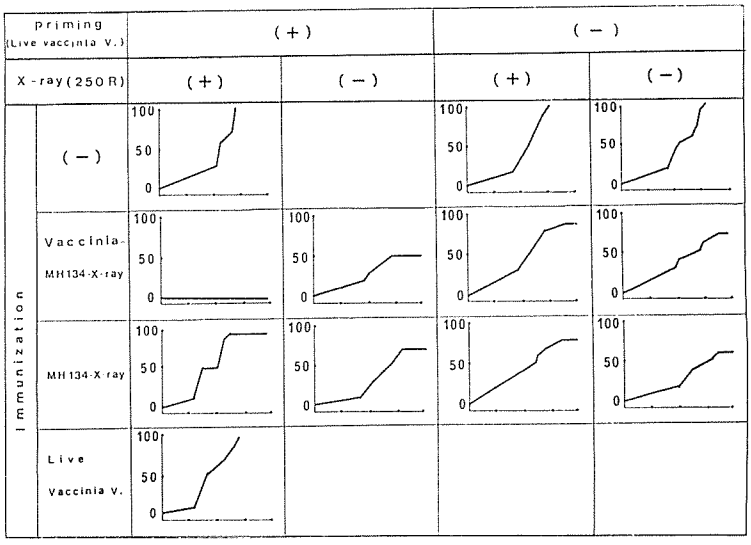

FIGURE 2. Cummulative mortalities of C3H/He mice after intraperitoneal challenge with 1×10^5 viable MH134 cells. Each group consisted of 10 mice. Mice were treated and immunized as described in the Materials and Methods.

DISCUSSION

Some viruses have oncolytic activity, which is simply due to viral multiplication in tumor cells. However, Lindenmann found that Erhlich ascites tumor-bearing mice survived intraperitoneal inoculation of influenza A virus (Lindenmann, 1962) and became immune to rechallenge with the same tumor cells (Lindenmann, 1965). He found that a lysate of tumor cells infected with influenza virus could elicit an immune response against the tumor cells; that is, a viral adjuvant effect enhanced tumor cell-specific antigens (Lindenmann and Klein, 1967), as in the helper antigen system reported by Mitchson (1970). After experiments in BALB/c mice, Wallack et al. (1977) reported success in treatment of cancer-bearing patients by immunization with a vaccinia oncolysate prepared from tumor tissue of the patients. In their mouse system they tested the immunoprophylaxic effect of a oncolysate derived from vaccinia virus-infected SV40-transformed mouse peritoneal macrophage tumor in 2 ways. One way was pretreatment of mice with the oncolysate only, and the other way was preimmunization of mice with vaccinia virus one week before pretreatment with the oncolysate. Preimmunization with vaccinia virus in the latter case, corresponded to priming in our system. They obtained equally good results in both ways. So, preimmunization with vaccinia virus may not influence immune systems, because, a period of one week is not long enough for induction of potent helper T lymphocytes. The mortality of mice, that were not primed with vaccinia virus, was decreased to only 70% in our system. It is not clear whether a vaccinia oncolysate is more potent for immunization of tumor cell-recipients than vaccinia virus-infected whole cells, because different types of tumor cells differ in malignancy.

Recent advances in tumor immunology have enabled us to manipulate immune systems concerning tumor rejection to some extent. Hamaoka et al. succeeded in inducing potent hapten-reactive helper T lymphocytes with striking augmentation of induction of tumor specific killer T lymphocytes by priming mice with 2, 4, 6-trinitrophenyl (TNP)-isologous mouse gamma globulin and by specifically inactivating TNP-reactive suppressor T cells with TNP conjugates of the nonimmunogenic D-amino acid copolymer, D-glutamic acid and D-lysine (D-GL) followed by immunization with TNP-haptenized syngeneic tumor cells (Hamaoka et al., 1979).

The present study was an extension of the studies of Hamaoka's group. Vaccinia virus has been used for more than a century to immunize billions of humans all over the world since Edward Jenner first introduced vaccination. Consequently, the virus had the advantage for future human trials that its reactions in humans are known.

As vaccinia virus is the biggest and the most complex enveloped virus, it should cause significant modification of the surface of infected cells. Miyamoto and Kato (1968 and 1971) reported that a vaccinia virus-induced antigen other than hemagglutinin was expressed on the cell surface in early stages of infection. Similar findings were reported by Ueda et al. (1972). Ikuta, Miyamoto and Kato (1981) showed that this antigen was a polypeptide with a molecular weight of about 40,000, and Ueda and Tagaya (1973) reported that it can induce cellular immunity in rabbits. Oie and Ichihashi (1981) found that a vaccinia virus-specific target antigen for recognition of anti-vaccinia virus cytotoxic T lymphocytes was formed on the surface of infected cells. These findings indicate that the generally weak immunogenic tumor associated transplantation antigen (TATA) (Klein, 1966) is strongly recognized together with vaccinia virus-induced antigen on virus-infected tumor cells in our system, because, without priming with vaccinia virus the immunization did not have any favorable effect against tumor challenge even in mice in which suppressor T lymphocytes were inactivated by X-ray irradi-

ation.

The present results indicate a more certain way of immunoprophylaxis against tumor than similar ways using viruses. Immunological analysis and similar experiments using solid tumors are in progress.

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