Tumor Immunity

Part 2. An Attempt at Immuno-Prophylaxis against Induction of DAB-Hepatoma

Michihiro Okuhara

First Department of Internal Medicine (Director: Prof. H. Ōkubo)
Kansai Medical School, Moriguchi, Osaka, Japan

Introduction

Takeda¹⁾ and Klein²⁾ demonstrated that tumor cells possess a specific antigen which does not exist in normal cells.

As already discussed in part 1³⁾, the problem, whether cross-reactivity among different tumors exists or not, is not yet settled, and hitherto negative results might be due to inadequate methods or techniques. Actually, as shown in part 1, active immunization with Yoshida Sarcoma (YS) cells markedly reduced the carcinogenesis induced by 2mg of 20-methylcholanthrene but could not prevent that induced by 10mg of the same drug, which quantity had perhaps blocked the immune response of the animals.

If there were a cross-reactivity among tumors, it may be possible to control tumor development by previous immunological treatment. In the present experiment, animals were immunized with YS or AH-130 and then their subsequent resistance against DAB-carcinogenesis was examined, the object being to see whether a cross-reactivity could be found between the immunizing tumor of long-term-transplantation and the tumor induced by DAB administration.

Materials and Methods

1. The animals: Donryu rats, 5 to 6 weeks old and weighing about 120g, were used.
2. Carcinogen: 3'-methyl-4-Dimethylaminoazobenzene (DAB) was used. DAB was mixed in 0.06% with the basal semi-synthetic diet (MF diet) of the Oriental Co.
3. Antigen: Yoshida Sarcoma and AH-130, both homologous to Donryu rats, were used. AH-130 is an ascites hepatoma induced by an azo-dye in the Sasaki Institute. YS is an ascites tumor which developed during the course of an experiment on chemical tumor induction. Its origin is not yet determined.

4. Experimental groups : The animals were divided in a control (no immunization) group and three immunization groups according to the antigen used, i. e. the YS, AH-130, and liver tissue immunization groups.

5. Preparation of antigens : The procedure employed by Aosaki⁴⁾ was adopted.

a) YS immunization group : YS cells obtained from the rats, 5 to 6 days after intra-peritoneal transplantation, were suspended in physiological saline (p.s.), the concentration of which was adjusted to 10^8 cells per ml (Solution I). An equal volume (1 ml) of 0.4mM. mitomycin C (MMC) in p.s. (Solution II) was added to the Solution I. The mixture (I+II) was incubated at 37°C for 3 hours. The YS cells thus treated with MMC were washed twice in p.s. at 2000 r.p.m. for 30 minutes. The packed cells were resuspended in p.s. so that its 0.5ml contained approximately 10^8 cells, and then used as antigen.

Fig. 1 Methods

b) AH-130 immunization group: AH-130 cells, similarly treated as the YS cells, were used as antigen.

c) Liver tissue immunization group: Liver tissue obtained from untreated rats, killed by ether, were minced as finely as possible with scissors. 0.2ml of the minced liver was mixed with 1ml of 0.4mM MMC in p.s. (Solution II) and then incubated for 3 hours at 37°C. They were then washed twice with p.s. at 2000 r.p.m. for 30 min.. The sediment was used for immunization.

6. Immunization procedure: Grouped rats received, at intervals of 2 weeks, 2 subcutaneous inoculations of 10^8 YS cells, 10^8 AH-130 cells, or 0.2ml of the sediment of treated liver tissue which should be equivalent of 10^8 tumor cells or more. Immediately after the second immunization, administration of 3'-Me-DAB was started. Then two subsequent inoculations at intervals of 2 months were performed.

Animals were killed by ether 4.5 or 5.5 months after the beginning of DAB administration and their livers were examined histologically by hematoxylineosin stain.

Results

Histological findings in the liver 4.5 and 5.5 months after the beginning of DAB administration are shown in Tables 1 and 2, respectively. The tumor incidence is summarized in Table 3 where it can be seen that 4.5 months after beginning of DAB administration, no significant difference in tumor incidence was found between the YS group (27%), the liver tissue group (30%), and the control group (27%), although the AH-130 group did not show any tumor development. But 5.5 months after the beginning of DAB, all three immunization groups showed a lower tumor incidence than the control

Table 1. Histological Findings 4.5 Months after The Beginning of DAB Administration

	Y.S. cells immunization group	AH-130 cells immunization group	Liver tissue immunization group	No immunization group
Number of rats	11	10	10	11
Normal	0	0	2	0
Liver fibrosis	5	8	3	6
Liver cirrhosis	3	2	2	2
Tumor	Hepatocellular type	1	0	1
	Cholangiocellular type	2	0	2
	Mixed type	0	0	1

Table 2. Histological Findings 4.5 Months after The Beginning of DAB Administration

	Y.S. cells immunization group	AH-130 cells immunization group	Liver tissue immunization group	No immunization group
Number of rats	10	11	9	10
Normal	0	0	0	0
Liver fibrosis	5	7	2	0
Liver cirrhosis	2	1	2	0
Tumor	Hepatocellular type	1	2	2
	Cholangiocellular type	2	1	2
	Mixed type	0	0	1

Table 3. Tumor Incidence

	Y.S. cells immunization group	AH-130 cells immunization group	Liver tissue immunization group	No immunization group
4.5 months after DAB administration	3/11 27%	0/10 0%	3/10 30%	3/11 27%
5.5 months after DAB administration	3/10 30%	3/11 27%	5/9 56%	10/10 100%

Denominators : total number of rats used for experiments

Numerators : number of rats which developed tumor

Table 4. Average Intake of DAB

	Y.S. cells immunization group	AH-130 cells immunization group	Liver tissue immunization group	No immunization group
4.5 months after DAB administration	1099mg	1064mg	1085mg	1027mg
5.5 months after DAB administration	1349mg	1307mg	1327mg	1320mg

group (100%) ; the AH-130 group (27%) showed the lowest tumor incidence, followed by YS group (30%) ; the liver tissue group, too, showed some reduction in tumor incidence (56%). Among these groups there was no remarkable difference in DAB intake, which is summarized in Table 4.

Discussion

The groups immunized with AH-130 or YS cells clearly showed resistance against DAB carcinogenesis at the 5.5th month. In this resistance obtained here, resistance caused by genetic difference (homologous immunity) between the tumor used as antigen and the immunized animals could not play a role, since transplantability was not applied as a marker of the experiment. As it has already been admitted, each organ or tissue has its individual specific antigenicity, apart from iso- or homologous antigens. Therefore, the possibility remains that the tumor cells as antigen might contain an organ specific antigen from its originating organ which might play a role in the induction of resistance against carcinogenesis, especially as AH-130 is a tumor originating from the liver. However, some investigators^{5) 6) 7)} have provided considerable evidence suggesting loss or decrease in the liver specific antigen in chemically induced hepatoma; for example, Weiler⁸⁾ utilizing the immunohistological procedure, and Baldwin^{9) 10)}, and Kalnins¹¹⁾ utilizing the agar gel double diffusion technique. Referring to those authors' results, the resistance obtained by AH-130 inoculation in my experiments does not seem to be derived from organ specific antigen. Likewise, the resistance in the liver tissue immunization group seems to be due to non-specific stimulation by the parenteral deposition of antigenic materials rather than immunization by the organ specific antigen. Therefore, it may possibly be concluded that the resistance against DAB carcinogenesis induced by the inoculation of AH-130 as well as YS depends on a common antigen, specific to tumors. The fact that, at the 4.5th month, the tumor incidence of YS immunization group showed no significant difference compared with that of the control group, although the former showed resistance (reduction of tumor incidence) at the 5.5th month, seems to suggest that the cross-reactivity between YS cells and DAB induced hepatoma is lower than that between AH-130 cells and DAB induced hepatoma.

The effectiveness of immunization against carcinogenesis, i.e. crossreactivity between the tumor used as antigen and the tumor induced by chemical carcinogen, has been already examined by some investigators. Takeda¹⁾ reported on the successful result of active immunization with insoluble lipo-protein of methylcholanthrene (MC) induced tumor against MC carcinogenesis in rats. Maisin¹²⁾, too, found a similar effectiveness of the microsomal fraction of DAB-induced hepatoma against tumor induction by DAB in mice. Usubuchi¹³⁾ indicated that the repeated injections of the same species, Usubuchi sarcoma, inhibits the development of 20-MC tumor in rats. On the other hand, negative results were reported by Prehn¹⁴⁾ and Klein²⁾, who immunized mice with MC-induced sarcoma and found no subsequent effectiveness against MC carcinogenesis. These negative results

could, conceivably, have been caused by inadequacy in method or technique, or by overdosages of carcinogen, which might block the immune response of the animals. In my above mentioned experiment, the resistance against DAB carcinogenesis was induced by pretreatment with MMC-treated tumor cells. Thus it has been demonstrated that the resistance against carcinogenesis can be induced by immunization using long-term-transplanted tumor as antigen. This result suggests that this method can be applied to clinical practice; if a tumor originating in one human is preserved, for example in a manner such as tissue culture, one could utilize it for immunization in case of need.

Summary

Liver carcinoma induced by DAB administration could be controlled by the active immunization of rats by inoculation with MMC-treated YS cells or AH-130 cells. The results obtained can be interpreted to mean that a DAB-induced hepatoma possesses the same tumor antigen as the tumor cells used for pretreatment, i.e. YS cells or AH-130 cells.

I wish to express my thanks to Dr. Y. Aosaki for his advice and encouragement.

(Presented at the 30th Annual Meeting of the Japanese Cancer Association, Tokyo, October, 1971.)

References

- 1) **Takeda, K.** : Autoimmunity in cancer-carriers, *Igaku No Ayumi*, **58**, 492, 1966. (in Japanese)
- 2) **Klein, E.** : Demonstration of resistance against methylcholanthrene-induced sarcomas in the primary autochthonous host, *Cancer Research*, **20**, 1561, 1960.
- 3) **Okuhara, M.** : Studies on tumor immunity : An attempt at immuno-prophylaxis against 20-methylcholanthrene-induced tumors, **23**, 24, 1971.
- 4) **Aosaki, Y.** : Tumor immunity, *The Journal of The Kansai Medical School*, **20**, 273, 1968. (in Japanese)
- 5) **Aizawa, K.** : Distribution in tissues and localization in cells of hemoagglutinin of histocompatibility (R antigen) in rats, *Igaku No Ayumi*, **17**, 515, 1967. (in Japanese)
- 6) **Potter, V. R.** : The biochemical approach to the cancer problem, *Feb. Proc.*, **17**, 691, 1958.
- 7) **Haughton, G.** : Tissue cell antigens : Antigens of mouse tumor cell ghosts, *Brit. J. Exp. Path.*, **43**, 488, 1962.
- 8) **Weiler, E.** : Antigenic comparison of normal and neoplastic tissues, *Acta Unio. Int. Contra Corcrum*, **15**, 929, 1959.
- 9) **Baldwin, R. W.** : Immunological studies on rat liver cell antigens during azo dye carcinogenesis, *Acta Unio. Internat. Contra Corcrum*, **19**, 545, 1963.

- 10) **Baldwin, R. W.** : Studies on rat liver cell antigens during the azo dye carcinogenesis, Brit. J. Cancer, **16**, 749, 1962.
- 11) **Kalnius, V. I.** : Loss of liver cell antigen in azo dye induced hepatomas of rats, Nature, **200**, 189, 1963.
- 12) **Maisin, J. H. F.** : Immunoprophylaxis of induced experimental hepatoma, Nature, **202**, 203, 1964.
- 13) **Usubuchi, I.** : Inhibitory effect of immunization on tumor production, Gann, **47**, 534, 1956. (in Japanese)
- 14) **Prehn, R. T.** : Failure of immunization against tumorigenesis, J. Nat Cancer Inst., **26**, 223, 1961.