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Studies on the Cellular Immunity and Enhancing Factors in MCA-induced Sarcoma in C_3H/HeN Mice*

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INTRODUCTION

The presence of tumor specific antigen(TSA) is supported by the experiments in which the growth of transplanted tumor cells is more effectively inhibited in the inbred mice immunized with the tumor cells than in the non-immunized(Foley, 1953; Prehn & Main, 1957; Sjögren, 1961; Habel, 1961; Morton, 1962; Weiss, 1964) and it is believed that most of the animal tumors are known to have the TSA (Foley, 1953; Prehn & Main, 1957; Sjögren, 1961; Habel, 1961, Morton, 1962; Weiss, 1964; Hellström, 1965; 1967ab; 1969abcd; 1970abc; 1971c; Attia, 1966; Barski, 1969; Bloom, 1969; Kronman, 1969; Le Francois, 1971; Halliday, 1972; Youn, 1973) as well as some human tumors are (Gold 1965ab, Häkkinen, 1966; 1974; Abelev, 1967; Hellström, 1968; 1979d; 1971ab; Eilber, 1970; Order, 1971; Morton, 1971; 1972).

On the other hand, the host bearing these antigenic tumors exhibits the defence mechanism by the cell-mediated immune responses with the help or interference of the humoral immune responses (Hellström, 1968; 1970d; 1971ab; Morton, 1971; 1972; Stewarts, 1971; Hayami, 1973; 1974; Tamerius, 1974; Nelson, 1975ab) and recent advances in cellular immunology have made it feasible to develop various in-vitro methods of measuring the cell-mediated immune responses to TSA quantitatively (Hellström, 1971d; Seeger, 1973; Cleveland, 1974).

To demonstrate cellular and humoral immune responses of the host against the MCA-induced sarcoma, the authors have carried out a preliminary study on MCA sarcoma induction in some inbred strains of mice, on the tumor grafts and on colony formation of the plated tumor cells in petri dish (Chang, 1975).

Here we report the continued results of works in which we could demonstrate the immune responses of C_3H/HeN mice by colony inhibition assay and transplantation method against MCA-induced sarcoma.

However, we gained some insights not only on the facts that the in-vitro peripheral action of enhancing sera from mice carrying advanced tumors was cytotoxic in the presence of complement, but also on the central blockade of immune peritoneal cells with enhancing sera or tumor antigen in the system we adopted.

And we discuss these observations with regard to the predictions of immunostimulation theory

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of tumor development(Prehn, 1971).

In addition, we report an experiment of transplantation method which used the minimal tumor cell dose as a challenge as well as an experiment which showed the existence of narrow dose range for immunoprotection of tumor with frozen-thawed tumor cell antigen.

Initial purpose of this work was to establish the methods to assay the cellular immunity of the host bearing experimentally induced tumors to be used as immunological parameters in measuring immune status of the host and it is considered to be partially fulfilled by this report and our in-progress works on MIF responses of peritoneal cells from mice in the various stages of tumor growth(Shin, 1976).

MATERIALS AND METHODS

Mice:

C_3H/HeN, $C_{57}BL$ and DDO mice which have been maintainted for 3 years by strict inbreeding at our laboratory were used.

The former two strains had been derived from the stocks at the Cancer Pathology Laboratory, Korean Atomic Energy Reaserch Institute, Seoul, Korea (Yun, 1973) and the latter one strain from stocks at the Department of Microbiology, Catholic Medical College, Seoul, Korea.

Tumors and cell culture:

We used one of the MCA-induced, transplantable sarcomas in C_3H/ HeN mice which had arisen 7-8 weeks following subcutaneous injection of 1 mg of 20-methylcholanthrene dissolved in 0.05 ml of lard (Foley 1953, Prehn & Main 1957), and which has been removed, minced, trypsinized, washed with isotonic phosphate-buffered saline(pH 7.4) twice aseptically and passaged by serial aniaml-passage at the dorsum of C_3H/HeN mice.

Most of the experiments were done with tumors of 9th or later passages in-vivo and it was cultivated in complete Waymouth's medium (GIBCO) which was supplemented with 30% fetal calf serum (DIFCO, not-heat-inactivated during this work), 1% of 100X Non-Essential Amino Acids (GIBCO), 1% 100 mM sodium pyruvate (GIBCO), 1% of 29.2% L-glutamine (GIBCO), 1.5% of 7.5% sodium bicarbonate (Merck) and 1% of antibiotics solution containing 20,000 U/ml penicillin G sodium (Keun Wha pharmaceutical Co. Ltd, Korea) and 20 mg of streptomycin sulfate (Hoechst, German), at 37°C, 5% CO_2, humidified incubator. (Hellström, 1971d)

Tumor cell antigen:

Trypsinized tumor cells were washed with isotonic phosphate buffered saline (pH 7.4), three times, cell counted with hemocytometer and stored frozen at -50°C till use. The frozen cells were thawed and appropriately diluted at the time of use.

Enhancing sera:

Mice carrying progressively growing tumor (more than 2.0cm in diameter) and the mice which were inoculated with the tumor cells with $5X10^4$ cells per mouse and which did not have palpable tumor mass at the injection site within the observation period of 3 months were bled by decapitation and designated as enhancing sera according to the biological activity(47) and immune sera arbitrarily, respectively. All the sera were sterilized by Milipore filtration and stored at −50°C.

Peritioneal cells:

These were obtained by the peritoneal washing technique of Barskie et al. (Barski, 1969; Le Francois, 1971; Youn, 1973).

7 ml of Hanks' BSS or Eagle's MEM containing 2 U/ml of heparin, 100 U/ml of penicillin and 100 mcg/ml of penicillin and 100 mcg/ml of streptomycin sulfate was injected into the

abdominal cavity of nonanesthesized mice with a multiperforated 18-gauge needle attached to a 10 ml syringe.

The abdomen was gently massaged and the fluid was aspirated and reinjected three times without removal of the needle.

The aspirated fluid was cooled to 0°C in ice-bath and washed with Hanks' BSS or Eagle's MEM containing 2 U/ml heparin twice and with complete Waymouth's medium once and the cell number were counted with the hemocytometer

The cell composition of peritoneal cells obtained from 25 mice has been studied with the help of Dr. K.Y. Song, Department of Pathology, College of Medicine, SNU and it consisted of average 54% lymphocyte and 45% macrophage cell type (from the reading of 100 slide prepared from peritoneal cell washings of 25 mice)

Colony inhibition assay:

This was carried out essentially by the methods of Hellström and Hellström (1971d). Trypsinized MCA tumor cell suspensions were diluted to make 2×10^4 or 3×10^3 cells in 10 ml of complete Waymouth's medium and seeded into each petri dish (90×15mm, Coring Pyrex) with 10 ml of diluted cell suspension and for the study of the serum-mediated effects on the plated target tumor cells(Table 5), 0.5ml of undiluted serum obtained from the experimental and control mice were added into petri dish. All the petri dishes were incubated at 37°C, 5% CO_2, humidified incubator for 24 hours.

After this incubation, the medium of the petri dish, containinng sera of experimental mice or control mice, was exchanged and at the same time the harvested peritoneal cells($5X10^6$ cells per petri dish) untreated or treated with serum or tumor antigen at 37°C for 30min (For the Experiment in Table 5) were added into the petri dishes.

At the end of further 24 hours' incubation, the culture medium was exchanged with fresh medium.

After another 3 days' incubation, the culture medium was decanted and the tumor cells were fixed with 95% methanol and stained with 0.5% aqueous crystal violet solution and the number of colonies was counted with the aid of arc projector (magnification about 45-fold).

Minimum number of MCA tumor cells necessary for establishment of tumor graft in strain of origin and on the fate in allogeneic host:

During the MCA-induced tumor passage at our laboratory, we found that 10^3-10^4 MCA tumor cells in vitro did form approximately 10-100 colonies (Table 3, 4, 5 and 8), but the same number of tumor cells cultivated in-vitro and injected subcutaneously into the normal C_3H/HeN mice of tumor origin was not tumorigenic during the period of observation (till 7 months of observation period) (Table 1). This means that, the mutation to non-tumorigenic transformation being unthinkable, the majority of the tumor cells cultivated in-vitro either became more immunogenic or the recipient normal host had some immunological surveillance mechanism normally operating. The latter holds true, even if the former statement is true.

In these experiments we had intentionally selected a tumor challenge dose($5X10^5$ or $1X10^5$ cells per mouse) which would give less than 100% tumor incidence in treated or untreated recipients, so that we could detect both resistance and acceleration of tumor graft.

Tumor allografts always failed, indicating the tumor cells have strong histocompatibility antigens of the C_3H/HeN. (Table 2)

Table 1. Minimum tumor cell dose for the tumor graft in strain of tumor origin with in-vitro cultured MCA-induced sarcoma cell suspensions

Cell dose	10^6	10^5	10^4	10^3	10^2	10^1
Exp. No. 1 Tumor incidence			3/4	0/5	0/2	
Exp. No. 2 Tumor incidence	3/3	2/2	0/6	0/4	0/5	0/4
Exp. No. 3 Tumor incidence	5/5	6/6	1/10	0/13	0/14	

Table 2. Tumor graft on allogeneic mice with MCA sarcoma cell (1×10^7 per mouse) induced at C_3 H/HeN

Mouse strain	DDO	C_{57}BL/6J	C_3H/HeN
Tumor Incidence	0/13	1/13*	10/10

* Tumor was found on a dead mouse and was completely autolysed at autopsy.

RESULTS

Effects of normal peritoneal cells on the colony formation of MCA sarcoma cells in-vitro:

Normal peritoneal cells from C_3H/HeN mice did not form colonies at our culture system, but increased the plating efficiency of the MCA-induced sarcoma cells significantly (about 15%, P-value=0.05) (Table 3), probably as a feeder layer effect.

Effects of peritoneal cells from immune mice on the colony formation of MCA-induced sarcoma cells in-vitro:

The immune peritoneal cells with single, double dose schedule of frozen-thawed tumor cells with 10^7 cells/mouse reduced the number of colonies, 32% and 37% respectively (P-value$<$ 0.05) (Table 4).

The immune mice were resistant to the in-vivo challenge of the same MCA tumor cells(5X 10^4 cells per mouse) (Table 3) Tumor incidence in the case of single dose schedule was 0%(0/7), and significant at P-value $<$0.01 and that of double dose schedule was 14.3% (1/7), and significant at P-value $<$0.1 when compared to the incidence of the control mice.

Variability in colony number in the immune

Table 3. Effects of normal peritoneal cells on the colony formation of MCA sarcoma cells

PC donor C_3H/HeN	Number of cells		Mean No. of colonies/plate(S.D.)	Plating efficiencies (%)
	Tumor cell	PC		
Normal	0	5×10^6(7)*	0	0
0	2×10^4	0 (4)	174±14	0.85
Normal	2×10^4	5×10^6(4)	200±6**	1.00

* Number of plate tested

** Significantly different from the colonies without peritoneal cells added, at P−value=0.05 by Student t-test.

Table 4. Effects of immune peritoneal cells on the colony formation of the MCA sarcoma cells

	PC donor C_3H/HeN	Number of cells		Mean No. of colonies/ plate(S.D.)	Plating efficiencies (%)	% inhibition
		Tumor cell	PC			
Exp. No. 1	Normal	2×10^4	5×10^6(4)	200±6	1.00	
	Immune*(single)	2×10^4	5×10^6(3)	135±9(1)	0.68	32.0
Exp. No. 2	Normal	2×10^4	5×10^6(3)	267±10	1.35	
	Immune**(double)	2×10^4	5×10^6(5)	168±42(2)	0.84	37.2

* Peritoneal cell obtained 4 weeks after i.p. injection of 10^7 MCA tumor cells (frozen-killed)

** Peritoneal cell obrained one week after the injection with 10^7 cells and the interval of immunization was 5 wks.

*** Number of plate tested.

(1); and (2); Significantly different from the normal peritoneal cell group at P-value <0.01 and <0.05, respectively by Student t— test.

Table 5. Tumor incidence of in-vivo challenge of MCA tumor cells (5×10^4 cells/mouse) in immune mice

	Control	Single-dose immunization	Double-dose immunization
Tumor incidence (%)	4/7 (57.1)	0/7* (0)	1/7** (14.3)

* Tumor challenge on week after single i.p. injection of frozen-killed tumor cells (1×10 cells/mouse) and significant at P-value <0.01 compared to the control by student t— test.

** Tumor challenge two weeks after last injection and mice were the same used in the experiment in the Table 2 and the difference with the control was significant at P-value <0.1 by student t— test.

peritoneal cell group with double dose schedule was remarkable (Table 4).

Effects of peritioneal cells from mice carrying tumors on colony formation of the MCA sarcoma cells:

Peritoneal cells from mice bearing 17 days-old small tumors (1 cm in diameter) inhibited colony formation of MCA sarcoma cells in-vitro approximately 19.5%, when compared to the data of the normal peritoneal cells, but showed a highly variable effects on number of colonies (Table 6). Experiment with peritoneal cells from mice bearing 10 days-old very small tumors(0.5 cm in diameter) was limited to one mice, but the reduction in number of colonies was dramatic (72.7% inhibition)

Peritoneal cells from mice carrying progressively growing tumor (2.0~3.0 cm in diameter, about 30-40 days old) showed slight, though statistically insignificant, increase in number of colonies which is 9.2% above the feeder layer effect seen in the data of normal peritoneal cell control group(Table 6).

Effect of sera on tumor cell graft in inbred mice:

From the all mice received tumor cell suspensions (1×10^5 cells per mouse) which had been mixed with sera from mice carrying progressively growing tumors and incubated for 30 min. at 37°C, tumor arose 100%.

The tumor incidences in mice group which received same tumor cell suspensions similarly treated with immune sera, phosphate-buffered saline, and normal sera were 30%, 40% and 55.5% respectively, but differences among these three groups were not significantly

Table 6. Effects of peritioneal cells from mice carrying tumors on the colony formation of the MCA sarcoma cells in vitro

	PC donor C_3H/HeN	Number of cells		Mean No. of colonies/ plate (S.D.)	Plating efficiencies	% inhibition
		Tumor cell	PC			
Exp. No. 1	Normal	2×10^4	5×10^6(4)*	200±6	1.00	
	Tumor bearer(1cm)	2×10^4	5×10^6(5)	161±47**	0.81	19.5
Exp. No. 2	Normal	2×10^4	5×10^6(3)	267±10	1.35	
	Tumor bearer(0.5cm)	2×10^4	5×10^6(1)	73	0.37	72.7
	Tumor bearer(3cm)	2×10^4	5×10^6(5)	292±8	1.41	−9.2

* Number of plate tested

** Individual No. of colonies tested were 91, 119, 177, 198 and 219.

different statistically. (Table 7)

Now we confirmed that cellular immunity, though not so strong, exists in mice immunized with frozen-thawed MCA tumor cell antigen or with viable timor cell graft using in-vitro and in-vivo methods, and that anti-tumor immunity expressed in-vitro as colony inhibition by peritoneal cell present in the mice bearing a small tumor declined at the time of progressive tumor growth, (Fig. 1) and that the sera from mice carrying the progressively growing tumors enhanced the establishment of same tumor cell graft in the recipient host in-vivo (Table 7).

Since such observations were not inconsistent with the phenomena of immunological eclipse described by Youn et al. (Barski 1969, Le Francois 1971, Youn 1973), we had attempted to confirm that the tumor antigen or sera of the tumor carrying host, themselves are involved in the immunological eclipse phenomena. So we carried out the following experiments.

Tumor incidence in the mice immunized with frozen-thawed tumor cell antigen and challenged with viable tumor cells:

As a first step, to obtain more efficient anti-tumor peritoneal cells than those used in experiments of Table 4, we inoculated C_3H/HeN mice with various doses of frozen-thawed tumor cells, weekly for 5 weeks, through the intraperitoneal route and challenging the immune mice with viable tumor cells (with 5×10^4 cells per mouse) two weeks after the last injection,

Table 7. Results of MCA tumor cell graft (1×10^5 cells per mouse) treated with various kinds of mouse serum

Serum donor(C_3H/HeN)	Normal	Tumor bearing (2cm in dia)	Immune	PBS control
Tumor incidence(%)	5/9	8/8**	3/10	4/10
	(55.5)	(100)*	(30)	(40)

Equal volume of indicated sera and tumor cell suspension (2×10^6/ml) were mixed and incubated at 37°C for 30 min. and injected subcutaneously into the dorsum of mice (C_3H/HeN) (0.1 ml/mouse)

* Significantly different from normal sera at P-value <0.01 by Student t− test.

** Among the 10 mice which had received the treated tumor cell suspensions, 3 mice became lethargic immediately after the injections (within one hour) and among them, one mice recovered, but two mice expired next day. We thought the symptoms might be antigen-antibody complex mediated, but the two dead mice excluded in the data.

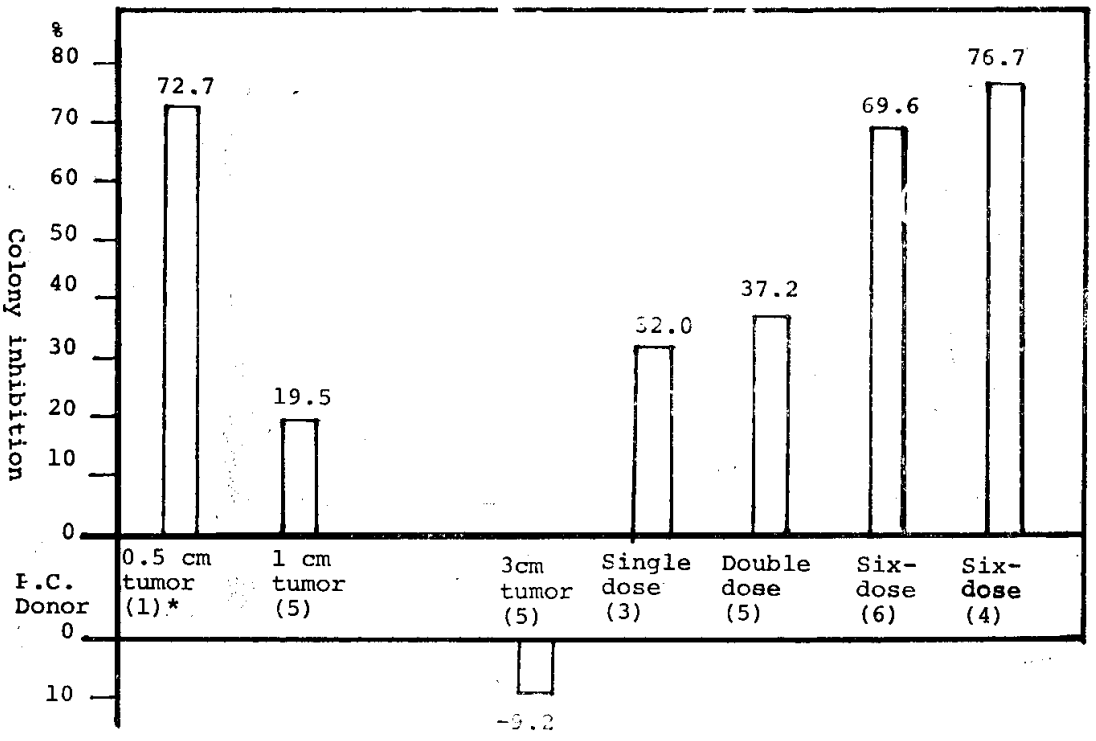

Fig. 1. Summary of the data of the colony inhibition assay described in Table 4, 6 and 8, according to the immune status of the peritoneal cell donor mice.
* represents the number of plates or mice tested.

the results represented diagramatically in Figure 2 have been obtained within the 3 months period of observation.

Tumor incidences in mice groups, immunized with 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, cells per mouse and in control mice, were 18.18% (2/11), 20% (2/10), 0%(0/10), 11.11% (1/9), 40% (4/10), 55.55%(5/9) and 38.1% (8/21) respectively.

For simplicity, summing up the two successive dose groups, the incidences of tumor would be 19.04 (4/21) for 10^3 -10^4 dose, 5.26% (1/19) for 10^5-10^6 dose, and 47.36% (9/19) for 10^7 -10^8 dose groups. The remarkable findings by Student t-test could be summarized as follows;

1. Difference in tumor incidences between the 10^3-10^4 dose group and the untreated control group was not significant.
2. Difference between 10^5-10^6 dose group and control was significant at the level of P-value <0.01.
3. Difference between 10^7-10^8 dose group and control group was not significant.

As a whole, immune mice with 10^5-10^6 frozen-thawed tumor cell antigen dose were most resistant to the tumor cell challenge and mice immunized with above and below these cell dose were not statistically different from the untreated control group.

Property of enhancing sera and the blockade of the immune peritoneal cell's reactivity with enhancing sera or with killed tumor cells in-vitro:

Using the peritoneal cells from mice sensitized intraperitoneally, weekly injections of 5×10^5 cells per mouse, according to the results of experiments in Figure 2, for 6 weeks at this time,

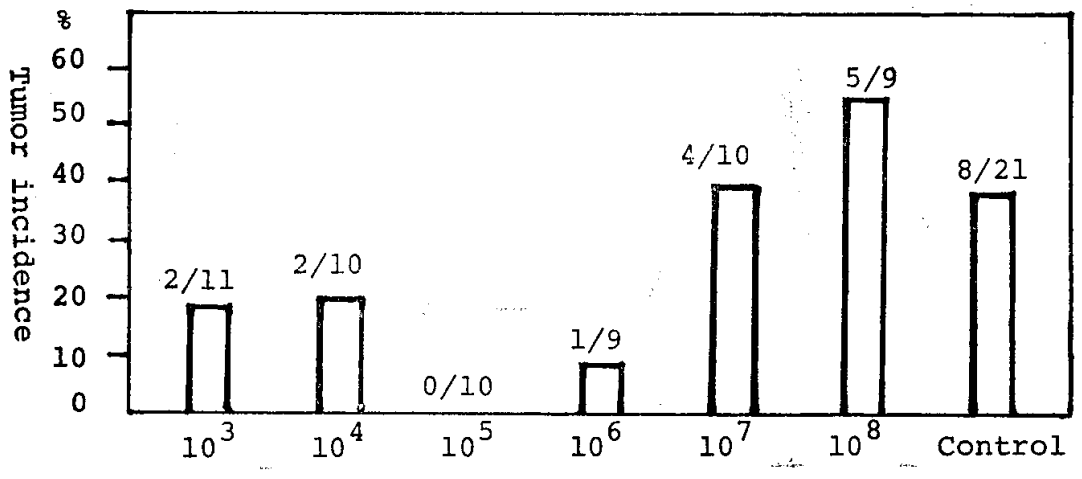

Fig. 2. Tumor incidence of in-vivo challenge of MCA-induced sarcoma cells (1×10^5 cells per mouse) into mice immunized with verying dose of frozen-thawed MCA tumor cells and into untreated control mice. Immunization was done weekly, I.P., for 5 weeks with indicated cell doses and the challenge was done two weeks after the last injection. P-value<0.1 by X^2-test.

we confirmed the consistent, and more efficient inhibition of colony formation by these immune peritoneal cells (69.6%, P-value <0.01, compared to the normal peritoneal cell group, Table 8) and by this result, we could ascribe the less efficient anti-tumor activity of immune peritoneal cells used in the experiments in Table 4 to the less efficient immunization protocol (one or two injectons with 10^7 cells).

Plated target tumor cells which had been exposed to the enhancing sera for 24 hours and which had been washed by exchange of medium before confrontation with normal or immune peritoneal cell were inhibited in number of colonies, 35.2% and 32.8%, respectively (Both P-value <0.05, compared to the normal sera and normal peritoneal cell groups and diffenrence between the normal and immune peritoneal cells groups was non-significant). This observation was against our expections derived from the data described in Table 7 and so is discussed in DISCUSSION.

Since the treatment of the sensitized peritoneal cells with enhancing sera or frozen-thawed tumor cell antigen abrogated the inhibitory activity of the immune peritoneal cells, approximately 98.3% and 98.3% respectively. (Table 8) (P-value <0.01, compared to the effect of normal serum-treated peritoneal cell group on the colony formation), these findings could explain some aspects of inactivity of peritoneal cells in the mice carrying advanced tumors (Table 5), in accord with data of others.

(Barski 1969, Le Francois 1971, Youn 1973, Hellström 1969cd, 1970ab, Hayami 1973, 1974)

And the treatment of plated target tumor cells with normal mouse serum did not influence the number of colonies (Table 8)

DISCUSSION

Confirming some basic facts on cellular and humoral effects of host to the various kinds of tumors established by several investigators by this work, we found that some immunological

Table 8. Effect of treatment of peritoneal cells on the colony formation of the plated MCA target tumor cells which have beeen treated or untreated

PD donor C_3H/HeN	Number of cells per dish				No. of colonies/ plate(S.D)	Plating efficiencies(%)	
	Tumor cell	Treatment	PC	Treatment			
Normal	3×10^3	None	5×10^6(6)**	None	38.5±11.7	1.28	Inhibition %
Immune*	3×10^3	None	5×10^6(6)	None	11.7±4.7(1)	0.39	69.6
Normal	3×10^3	NS	5×10^6(7)	None	33.6±9.2	1.12	inhibition %
Normal	3×10^3	ES	5×10^6(4)	None	21.8±4.7(2)	0.73	35.2
Immune	3×10^3	ES	5×10^6(5)	None	22.6±4.2(2)	0.75	32.8
Immune	3×10^3	None	5×10^6(4)	NS	9.0±1.8(1)	0.30	abrogation %
Immune	3×10^3	None	5×10^6(6)	ES	34.5±10.6(3)	1.15	98.3
Immune	3×10^3	None	5×10^6(7)	TA	34.4±10.0(3)	1.15	98.3

NS; normal mouse serum(C_3H/HeN), ES; enhancinge srum, TA; frozen-thawed tumor cell antigne'

* Immune schedule was i.p., weekly for 6 weeks at the cell dose of 5×10^5 frozen-killed cells per mouse.

** Number of plates tested

(1); Significantly different from the data of normal control peritoneal cell group at the level of P-value <0.01 by Student t− test

(2); Significantly different from the data of normal serum treated target cell+normal peritoneal cell group at the P-value <0.05

(3); Significantly different from the data of normal serum treated immune peritoneal cell group at the P-value <0.01.

The peritoneal cells used were pooled from the mice of the same experimental groups and treatment of the immune peritoneal cells with indicated sera or antigen was carried out as follows; equal parts of peritoneal cell suspension and sera (undiluted) or tumor antigen (PC: killed tumor cell$=5\times10^6 : 5\times10^7$ ratio) were mixed and incubated at 37°C for 30 min., and washed with complete Waymouth's medium twice before adding the cells into petri dish.

defence mechanism is operating in tumor grafts, which is domonstrated by the failure of the transplanted tumor cells(10^3-10^4 viable cells per mouse) to grow in normal mice, although these cells grow to form colonies in the range of 10-100 counts in-vitro-in concordance with the predictions of immunological surveillance theory of Burnet (1970).

The situation of the inoculation of a small number of tumor cells and the nature of the ability of normal mice to reject them is recently touched by Greenberg(1976) and if the situation is an adequate model for an oncogenic mutation in normal host and if the immune surveillance of tumor exists, he favors the possibility that it functions as a thymus-independent, non-adaptive phenomenon rather than Burnet's thymus-dependent adaptive mechanism, with the evidence of the absence of memory response of the mice inoculated with a small number of tumor cells($10^3\sim10^4$ dose groups in Fig. 2).

The immunity developing in mice presensitized by single or double doses of intraperitoneal injections of the frozen-thawed MCA tumor cells could be detected by the colony inhibition assay in-vitro by use of peritoneal cells as effector cells from the immune mice and by the tumor cell transplantation to the same mice in-vivo.

Although the evolution of the cellular immunity at early stage of tumor cell inoculation is not clear in this study due to the limited sample size, the findings on the decreased cytotoxic activity of the peritoneal cells from the mice bearing huge tumors agree well with the

data of Youn et al. (Barski, 1969; Le Francois, 1971; Youn, 1973) who, using also the colony inhibition assays, reported an immunological "eclipse" period observed regularly in mice bearing tumors ≦1 cm in diameter and we found that the sera obtained from the same mice enhanced dramatically the establishment of tumor cell grafts and so we called the sera enhancing sera as others did.

To define more efficient immunization schedule with frozen-thawed MCA tumor cells and to analyze the activites of the sensitized cells and enhancing sera in detail, further study was undertaken.

From the in-vivo tumor challenge experiments on the mice injected weekly, i.p., with various tumor cell doses per mouse for 5 weeks, essential conclusions to be drawn are that a narrow zone of tumor cell antigen dose per mouse exists for optimal in-vivo sensitization and above and below these cell number the anti-tumor immunity decreased, showing that the diagram in Figure 2 is similar to the phenomena of the low zone and high zone tolerance in the situation of immunization with weak antigens.

Moreover, this finding might reflect one aspect of complex immunological situations in the host bearing tumors, such as the tumor incidences in the cases of immunization with low doses of frozen-thawed tumor cells (10^3-10^4) might reflect a malignant tumor cell developing in the host which is inadequate for immunologic capacity to reject the nascent tumor and in the cases of immunization with high doses of frozen-thawed tumor antigen (10^7-10^8) might reflect the situation in the host bearing tumors ≧1 Cm in diameter in which the immunological "eclipse" is observed regularly (Table 5 and data of Youn, 1973; Thomson, 1975; Grosser, 1976).

Anyway, if this finding in MCA-induced sarcoma extends to other tumor systems, it would be considered to be important from the practical point of view in researches on the immunological manipulation of the host with tumor antigen, such as tumor vaccine which might be developed in the future (Prager 1973, Ray 1976) and further study on the mechanism operating in these observations would be desirable.

Similar dose dependence of immunoprotection of tumor has been reported by Baldin (1973) with irradiated tumor cell and Pellis (1975) with 3M KCI solubilized tumor cell antigen (Vaage 1972, 1973).

Confirming the not-inconsistent results, reached by several investigators, in which the blocking factors in sera from tumor-bearing mice might be antigen-antibody complex, capable of binding to the target cells and/or reacting lymphocytes immune to their antigens, thus blocking the lymphocytes' reactivity, which was demonstrated in this work by the blocking of the activities of the immune peritoneal cells by the confrontation with frozen-thawed tumor cell antigen or enhancing sera before being added to the plated target tumor cells, we found paradoxical results between activities of the same batch of enhancing sera from mice carrying advanced tumors in in-vivo experiment of tumor transplantation and those of the in-vitro colony inhibition assay experiment.

The in-vitro observations could be explained by assumption that the cytotoxic antibody in the enhancing sera with not-heat-inactivated, endogenous complement (MATERIAL AND METHOD) in Fetal Calf Serum in culture medium could reduce the number of colonies in-vitro and if this argument is correct, although evidences of possible existence of such situation are already presented by several investigators (Hayami 1973, 1974, Tamerius 1974), except that the incubation time of the plated target

tumor cells with enhancing sera is prolonged in this study, the same cytotoxic humoral factors inoculated with tumor cells in mice in the presence of complement might kill the inoculated tumor cells and might help the mice reject the tumor cells.

But the finding in-vivo experiment as mentioned in RESULTS was that, against our expectations, the same humoral factors in the serum enhanced the tumor development in mice. Since the enhancing sera, capable to block centrally the reactivity of immune peritoneal cells in-vitro(Table 7), were paradoxically able to kill the plated target tumor cells, when the incubation with the sera was prolonged for 24 hours in the presence of complement, it is obliged to suppose and propose that, if the in-vitro results could be applied directly to the understanding of in-vivo experiment of ours, the sera acting in-vivo as an afferent and/or central blockade of regionally infiltrating immunocompetent cells, the sublethally damaged cells or remaining viablecells escaped from the attack of the cytotoxic humoral factor were stimulated to divide to form a tumor mass.

Such a situation does grossly agree with the predictions of the immunostimulation theory of tumor development (Prehn, 1971) which postulates that the lesser degrees of immune reactivity may promote the growth of nascent tumor and that an immune reaction may at times produce better tumor growth than would occur in the total absence of immune reactivity.

It would be a highly possible mechanism in enhancement phenomena that cytotoxic activity in the enhancing sera demonstrated in our system is not destructive but constructive on tumor graft.

Perhaps the most important conclusion to be drawn from the foregoing is that the tumor growth would be a vector sum of the B-and T-cell interplays, and the circulating tumor antigen or immune complexes may play an important role in the tumor-host relationship through their interference with cell-mediated immunity.

SUMMARY

Colony inhibition assay in-vitro using the peritoneal cells as the effector cells and in-vivo transplantation method were adopted to demonstrate cellular and humoral immunity to MCA-induced sarcoma in tumor-bearing and in mice immunized with various doses of MCA tumor antigen.

Transplantation of MCA sarcoma in normal immune strain of tumor origin and in allogeneic mice revealed that some immunolocgial surveillance mechanism is operating normally.

Peritoneal cells from immune mice consistently inhibited colony formation of plated target tumor cells in-vitro, and the confrontation of the peritoneal cells with tumor antigen or sera from mice carrying advanced tumors, before the treated peritoneal cells being added to plated target tumor cells, abrogated the anti-tumor activities of those cells.

Peritoneal cells from mice bearing advanced tumors were found inactive in the inhibition of colony formation in-vitro and although the sera from the same mice enhanced dramatically the tumor graft establishment in-vivo, it was destructive on the plated tumor cells in-vitro.

The doses of killed tumor cells on which the immunoprotection of the host against the same viable tumor cells was dependent were investigated with in-vivo challenge method and a

narrow zone of tumor antigen dose for efficient protection was found to exist in the 5-weeks' schedule of immunization with one-week interval and via I.P. injections.

≫國文抄錄≪

MCA誘發肉腫에 있어서의 細胞性 免疫과 增強要因에 關한 硏究

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腫瘍에 對한 宿主의 細胞性 및 體液性 免疫反應을 測定하기 위하여, C_3H/HeN마우스에 메칠콜란스렌으로 誘發시킨 肉腫에서 腹腔細胞를 作動細胞로 使用한 集落抑制法과 最小 腫瘍細胞量을 使用한 腫瘍移植法을 使用하여 다음과 같은 成績을 얻었다.

1. 腫瘍抗原으로 免疫된 마우스에서 얻은 腹腔細胞는 標的腫瘍細胞의 集落形成을 恒常 抑制하였으며 이 免疫腹腔細胞를 腫瘍抗原이나 漸進的으로 成長하는 肉腫을 가진 宿主의 血淸과 먼저 處理하고난 다음 標的細胞에 添加하였을 때는 이 免疫腹腔細胞의 集落抑制力이 完全히 除去되었다.

2. 漸進的으로 成長하는 腫瘍을 가진 宿主로부터 얻은 腹腔細胞는 腫瘍의 크기가 적을 때 存在하던 腫瘍集落抑制力이 없었으며 이 宿主의 血淸은 同一腫瘍細胞의 移植率을 顯著히 增强시켰으나 生體外 集落抑制法에서는 腹腔細胞供給마우스의 免疫狀態와는 關係없이 標的腫瘍細胞를 죽였다.

3. 零下 50°C에 冷凍保存한 腫瘍細胞抗原을 使用하여 五週間, 每週 腹腔內 注射하여 免疫한 마우스群에서는 每回 마우스當 10^4—10^5의 腫瘍細胞抗原이 腫瘍移植免疫獲得에 가장 適合한 量이었다.

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