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RESEARCH ON EXPERIMENTALLY INDUCED CERVICAL CANCERS AND THE REACTIVITY OF SPLEEN CELLS TO CON A

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Synopsis We have investigated the spleen cell response to Con A in terms of the blastoformation response of lymphocytes, which is known to be an index of the nonspecific immune response of the host organism, in various stages of experimentally induced lesions of the murine uterine cervix. It was found that, under the controlled conditions of the present experiment, suppressor T cells are induced in histological samples exhibiting AT III or severer lesions, which correspond to carcinoma of the uterine cervix. This fact suggests the possibility that antibody synthesis is suppressed. In animals administered the immunopotentiator, ATSO, the development of malignant lesions of the uterine cervix was suppressed, the induction of suppressor T cells was suppressed and the response of spleen cells to Con A was found to decrease.

Key words: Cell mediated immunity • Spleen cell • Cervical cancer • Suppressor T cell

Introduction

It is of great importance to determine the nature of the host's immune response at various stages of cancer of the uterine cervix. The current research was undertaken to measure one of the non-specific immune responses, the blastoformation response of lymphocytes and to determine at which stage significant changes in this response occur. Furthermore, in order to determine the effects of administered immunopotentiator, histological and immunological studies, i.e. in terms of the blastoformation response of lymphocytes, were made on the cervical tissue following immunopotentiator administration and its effectiveness in preventing such lesions was investigated.

Many reports of experimentally induced cervical cancers have appeared since Cioli's study in 1929. In the present study we have used the experimental model of Taki and Iijima for producing cervical cancers in the mouse within a brief period and with a high incidence by application of 20-methylcholanthrene (20-MC) by means of a cotton thread inserted into the murine uterine cervix. It is of particular interest in such studies to determine the nature of the changes in the spleen cell response to Concanavalin A (Con A)[7] at various stages of the development of such lesions. We have therefore studied the correlation between the histology of the cervical tissue obtained weekly between 1 and 12 weeks following insertion of a 20-MC thread and this response in the excised spleens in terms of the ^{3}H-thymidine (^{3}H-TdR) uptake by the spleen. ATSO[4] from Coriolus versicolor Iwade, as an immunopotentiator, was administered and the differences in incidence between treated and non-treated groups examined. Finally, investigation was made of the effects of this immunopotentiator on the spleen cell response to Con A.

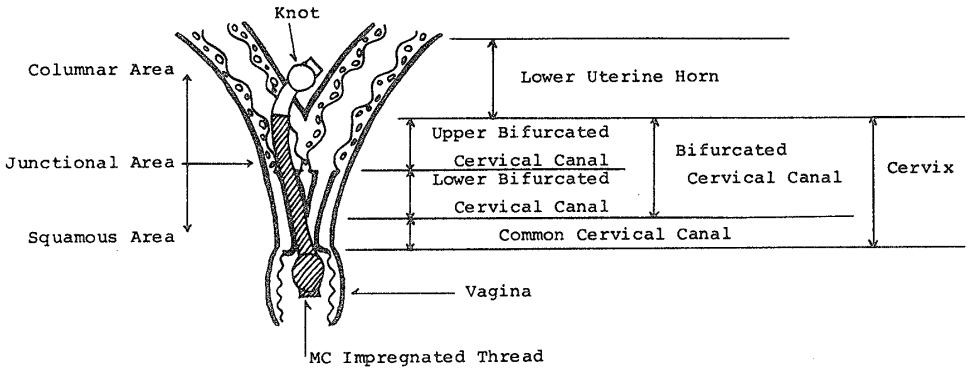
Experimental Methods and Results

1) Experiment I. Experimental induction of uterine cervical cancer in the mouse.

Mature female ddY mice roughly 10 weeks old were used. A total of 480 mice had the 20-MC thread implanted. Excluding all animals which died within a few days of implantation or, at autopsy, in which the thread had become removed, 309 animals were used in the study.

Method. Experimental cancers were produced using Taki and Iijima's method[9] for 20-

Fig. 1. Schematic drawing of thread application to mouse uterine cervix



MC thread insertion (which is an improvement on Murphy's method for producing uterine cervical cancer) under the administration of ether anesthesia. That is, a 15 cm piece of No. 20 cotton thread is cut and knotted at one end. After 10 hrs rinsing in water, it is soaked in alcohol ether. Next, 20-MC and bee's wax (in a ratio of 1:3 by weight) are heated and roughly 7 mm from the knotted end are then soaked in the solution. Finally, the 20-MC thread is inserted through the eye of a dull needle and the thread lead from the external os of the uterus to the cervidal canal and the needle is extracted from the lower uterine horn. The knotted end is thereby put in direct contact with the external os. The other end is then secured at the external uterine wall and the abdomen is sutured (Fig. 1).

Animals were sacrified between 1 and 12 weeks following 20-MC thread insertion and histological examination done only in those animals in which the thread was found to remain in the cervical region. A 10% neutral formalin solution was used for fixation. In addition to Taki's classification[9], into 4 groups normal, (photo 1); nonatypical hyperplasia, (photo 2, 3); and atypical hyperplasia (AT), (Fig. 2), subdivision of AT into AT I, (photo 4), AT II, (photo 5) and AT III, (photo 6) and invasive cancer (IC), (photo 7) were used. Similar experiments were also performed in animals administered the immunopotentiator (IP), ATSO. IP was administered subcutaneously in doses of 100 mg/kg twice weekly for the entire period from thread insertion until sacrifice.

Photo. 1. Normal

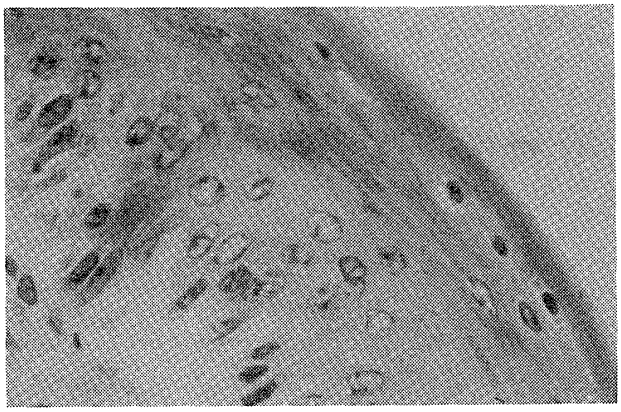
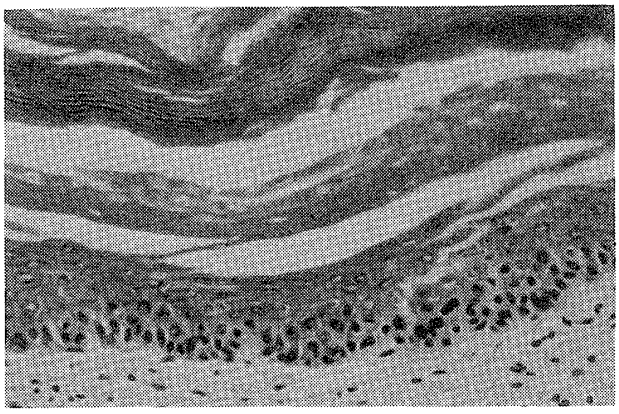


Photo. 2. Hyperkeratosis



Among the 199 animals not administered IP, the incidence of nonatypical hyperplasia decreased with time, whereas those with AT II or severer lesions increased. At III first appeared 4

Photo. 3. Squamous metaplasia

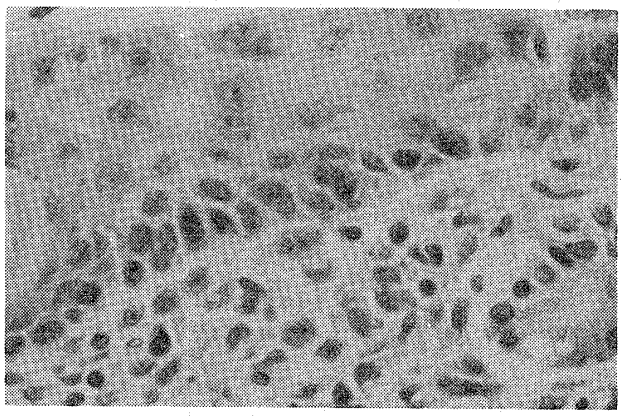


Fig. 2.

AT I: Lesion limited to less than 1/2 of the epithelium
AT II: Lesion extending over 1/2 of the epithelium
AT III: Lesion extending to all layers of the epithelium

Photo. 4. Atypical hyperplasia (AT) I

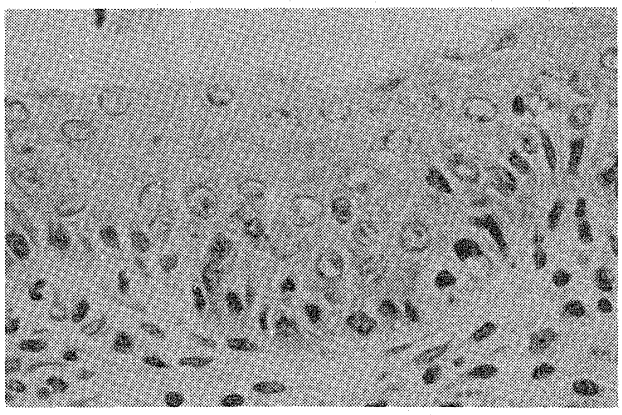


Photo. 5. Atypical hyperplasia (AT) II

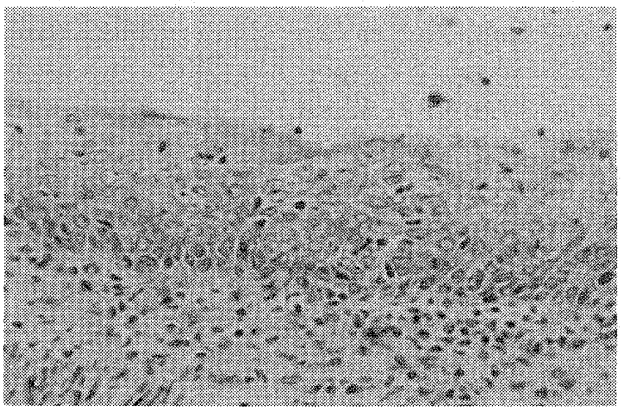


Photo. 6. Atypical hyperplasia (AT) III

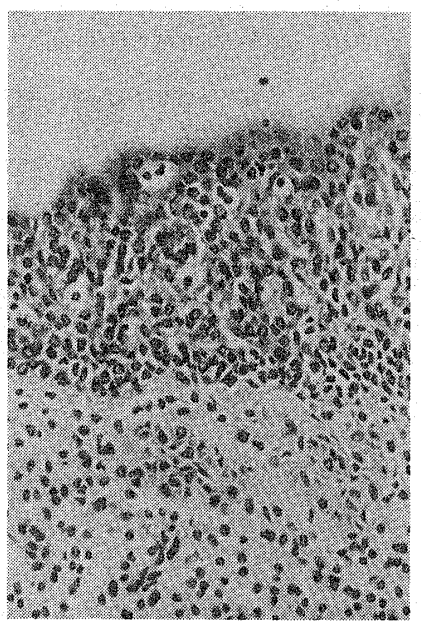
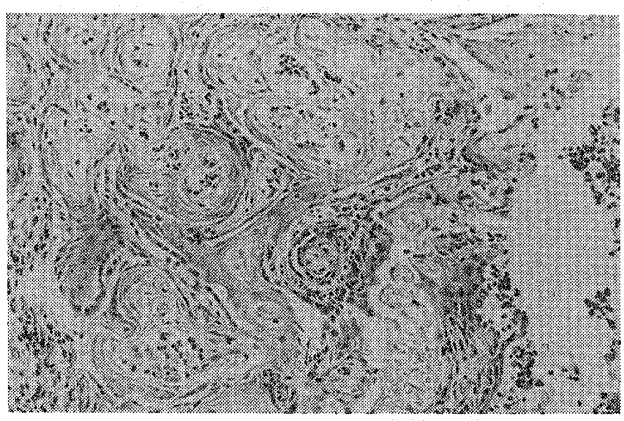


Photo. 7. Invasive cancer



weeks after thread insertion, and IC first appeared after 9 weeks. After the 9th week, the majority of animals had lesions at least as severe as AT I (Fig. 3).

Among the 110 animals administered IP, the majority had histological pictures less severe than AT I. Malignant lesions (AT III or IC) were found in only 2 of the animals autopsied after 7 weeks (Fig. 4).

2) Experiment II. The correlation between the histological severity of murine cervical lesions and the spleen cell response to Con A[5].

Fig. 3. Weekly incidence rate of normal, nonatypical, atypical and invasive carcinoma in cervical area of mouse uterus after application of MC thread

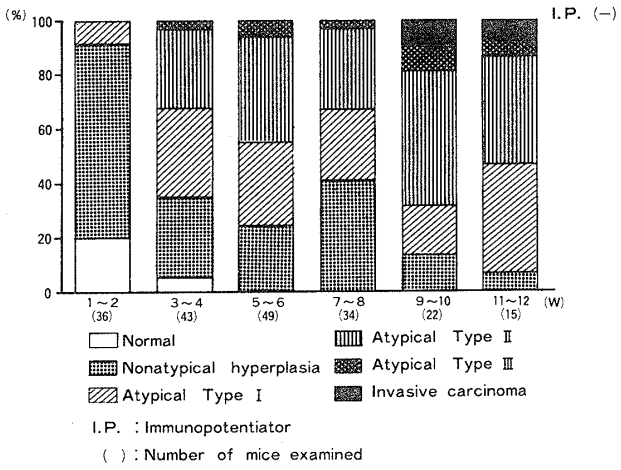
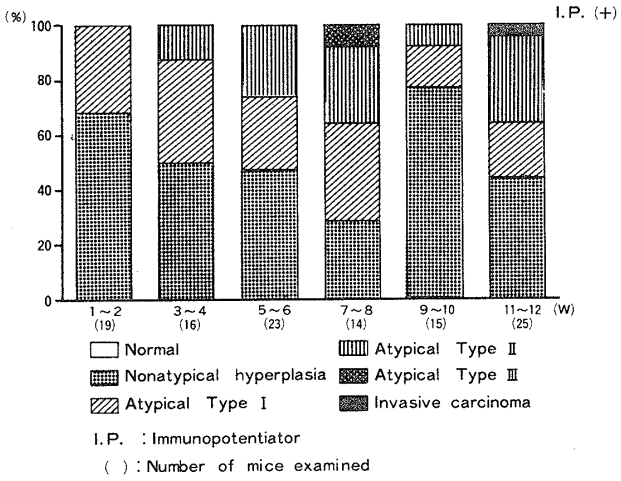
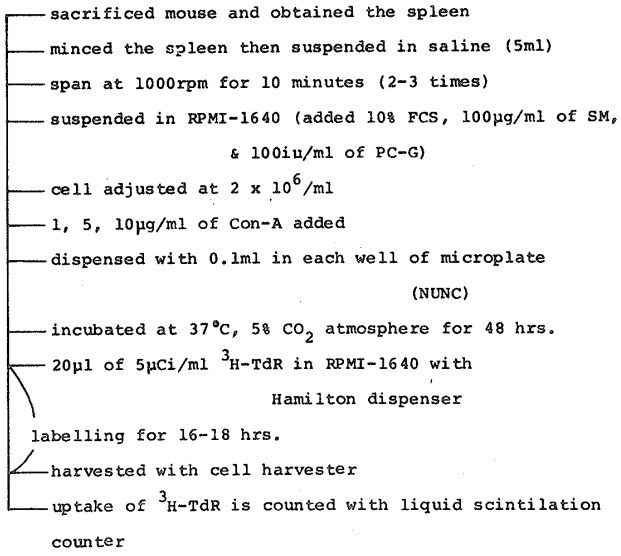


Fig. 4. Weekly incidence rate of normal, nonatypical, atypical and invasive carcinoma in cervical area of mouse uterus after application of MC thread



Method. The uterus and spleen were excised from ddY mice in which 20-MC threads had been inserted. Histological examination of the cervical region of the uterus was performed. The excised spleen was minced in physiological saline. Next, washing of the spleen cells was undertaken by thrice centrifuging the cells in physiological saline for 10 minutes at 1000 rpm. The spleen cells were then suspended in a medium of 10% Fetal Calf Serum (FCS), 100 μg/ml SM and RPMI-1640 with 100 IU/ml PC-G added. The cell count was adjusted to 2

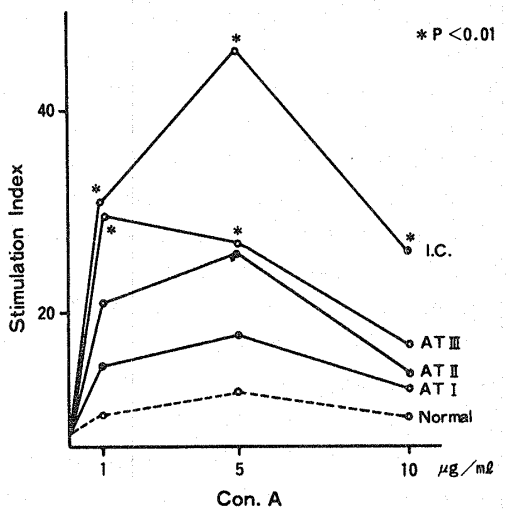
Fig. 5. Con-A method



$\times 10^6$/ml and 0.1 ml added to microplates to which 0.01 ml of Con A at concentrations of 1, 5, or 10 μg/ml were added. Each plate was then cultured for 48 hrs at 37.0°C in 5% CO_2 and ^{3}H-TdR 5 μCi/ml added. After 16-18 hrs of labelling, the cells were harvested with a cell harvester and the ^{3}H-TdR uptake measured by a liquid scintillation counter (Fig. 5). All experiments were performed in triplicate. The ^{3}H-TdR uptake was expressed in terms of a stimulation index (SI). The SI of a normal cervical histological sample was taken as a control, and the SI of each of the histological samples to which 1, 5 or 10 μg/ml of Con A had been added were compared.

As shown in Fig. 6, the SI of the histological samples from AT I to IC and of the samples to which increasing concentrations of Con A were added showed increasingly high SI values. That is, the spleen cell response to Con A increased in the order AT I, AT II, AT III and IC. Comparison of the responses of the control and AT III tissue samples showed that the spleen cells response to Con A was significantly greater than control values in concentrations of Con A equal to 1 or 5 μg/ml. Comparison of control and IC tissue revealed significantly greater responses in the IC tissue at all concentrations of Con A.

Fig. 6. Stimulation Index in various histological types



3) Experiment III. Spleen cell response to Con A in animals administered or not administered IP.

Method. 20-MC threads were inserted into the cervical region of ddY mouse uterus in order to produce neoplastic lesions and IP was subsequently administered. The IP was ATSO, which is a polysaccharide derived from Coriolus versicolor Iwade. IP was administered subcutaneously twice weekly in doses of 100 mg/kg from the time of 20-MC thread implantation until sacrifice. Uteri and spleens were excised from the sacrificed mice and histological examination of the uterine cervix undertaken. The spleen cell response to Con A was determined in the excised spleen tissue and comparison made with that in mice not given IP, according to each histological type. It was found that IP administration resulted in suppression of development of malignant cervical cancers, details concerning which were noted under the section concerned with Experiment I. There was also a tendency for the spleen cell response to Con A to be reduced in the IP administered animals with nonatypical hyperplasia (Table 1), AT I (Table 2), AT II (Table 3), AT III (Table 4) and IC (Table 5) lesions.

Discussion

From the above experimental results, it is ap-

Table 1. The Stimulation Index at various concentrations of Con.A in nonatypical hyperplasia

		Exper. No.	1	5	10 (μg/mℓ)
I.P.	(−)	52	10.72 ±2.694	19.51 ±3.678	9.16 ±2.252
	(+)	51	6.05 ±0.709	8.31 ±1.624	4.31 ±0.617

I.P.: Immunopotentiator (Mean ± S.E.)
(As I.P., ATSO was used)

Table 2. The Stimulation Index at various concentrations of Con.A in AT I

		Exper. No.	1	5	10 (μg/mℓ)
I.P.	(−)	40	9.48 ±1.767	13.96 ±2.300	6.79 ±1.114
	(+)	29	5.39 ±0.901	7.13 ±1.308	3.67 ±0.561
				P<0.05	P<0.02

I.P.: Immunopotentiator (Mean ± S.E.)
(As I.P., ATSO was used)

Table 3. The Stimulation Index at various concentrations of Con.A in AT II

		Exper. No.	1	5	10 (μg/mℓ)
I.P.	(−)	48	17.07 ±6.907	23.00 ±6.414	8.49 ±2.237
	(+)	19	5.50 ±1.165	6.80 ±1.259	3.97 ±0.570

I.P.: Immunopotentiator (Mean ± S.E.)
(As I.P., ATSO was used)

Table 4. The Stimulation Index at various concentrations of Con.A in AT III

		Exper. No.	1	5	10 (μg/mℓ)
I.P.	(−)	7	27.91 ±7.236	24.24 ±5.449	11.98 ±4.326
	(+)	1	2.24	6.22	3.03

I.P.: Immunopotentiator (Mean ± S.E.)
(As I.P., ATSO was used)

Table 5. The Stimulation Index at various concentrations of Con.A in I.C.

		Exper. No.	1	5	10 ($\mu g/m\ell$)
I.P.	(−)	3	28.90 ±10.283	46.97 ±9.860	22.23 ±7.448
	(+)	1	0.83	1.13	1.00

I.P.: Immunopotentiator (Mean ± S.E.)
(As I.P., ATSO was used)

parent that the development of lesions of the uterine cervix in the mouse of AT II, AT III or IC severity is suppressed due to the administration of the immunopotentiator, ATSO. Furthermore, in comparison with control animals, the spleen cell response to Con A was significantly increased in AT III lesions, which corresponds to carcinoma in situ. This response was also significantly increased in IC lesions. Administration of IP was found to significantly decrease the spleen cell response, as compared to the response in non-treated animals.

It is generally held that T-cells play a major role in the regulation of the immune response, most important of which are the helper T cells and suppressor T cells[6]. In experiments using rats, Tada et al.[8] have demonstrated the presence of suppressor T cells in many immune response phenomena. Using mice, Benjamin[1] showed the presence of suppressor T-cells which suppress antibody production in spleen cells. It has also been reported that in the mouse Con A stimulates T cells and induces suppressor T cells. In contrast, Gershon et al.[2][3] demonstrated the presence of suppressor T cells among mouse spleen cells and also showed the presence of helper T cells. From those results, they concluded that, using their experimental system, the functioning of either suppressor T cells or helper T cells can be induced. In the current study using mice, it was predicted that the results discussed earlier would be obtained using Con A as mitogen, in light of the results of Gershon et al. That is, suppressor T cells among spleen cells were induced by Con A in mouse uterine cervical tissue with IC lesions. Consequently, it is thought that the response was greater than that in the controls. Furthermore, in the animals administered ATSO, development of malignant lesions of the uterine cervix was significantly suppressed and the response of spleen cells to Con A in these animals was indeed low. This low response is thought to be due to the suppression of induction of suppressor T cells by ATSO administration. As a consequence, antibody production increases and the development of malignant lesions of the cervix decrease. In the present experiments, the above-mentioned results could be obtained under the given conditions. Henceforth, further studies need to be undertaken under different conditions.

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概要 マウス子宮頸癌発生段階における，宿主の免疫能，今回は非特異的免疫の指標の1つであるリンパ球幼若化反応を，Spleen cell と Con A の関連性において検討した．その結果，今回の一定条件下における実験では，子宮頸部上皮内癌に相当する Atypical hyperplasia Ⅲ以上の組織型を示すものは suppressor T cell の誘導をもたらし，抗体産生を抑制した可能性を推測させた．また，免疫賦活剤としての ATSO の投与は，子宮頸部悪性病変の発生を抑え，suppressor T cell の誘導を抑制し，Spleen cell の Con A に対する反応性を低下させる傾向を示した．