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ADJUVANT IMMUNOTHERAPY WITH ANTI-TUMOR STR. PYOGENES PREPARATION (IMMUNOPOTENTIATOR OK-432) IN UROGENITAL CARCINOMA

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As a curative means for urogenital carcinoma, non-specific immunotherapy is now standing in the spotlight, in addition to such measures as surgical operation, chemotherapy and radiation-therapy.

We applied an anti-tumor streptococcal pyogenes preparation (OK-432) as an adjuvant (immunopotentiator) to 54 cases principally with urogenital carcinoma through intracutaneous injection in order to examine its immunostimulative activity.

Items examined include counts of leucocyte and lymphocyte in the peripheral blood, delayed hypersensitivity skin tests to PPD and PHA, PHA and Con. A-induced blastogenesis, T. B. cell counting and IgG-FcR+ T cell.

As the results, this intracutaneous injection method was found to be simple and to have caused no side reaction, further preventing leukopenia which usually occurs after chemotherapy. It was found to be effective in preserving normal cellular immunity, thus working as an agent contributing to the prolongation of life of the host.

INTRODUCTION

The non-specific immunotherapy has recently been used more and more widely in the treatment of the carcinoma, and it is one of the anti-tumor therapeutic means which are expected to be effective. The immunotherapy is considered to exert its anti-tumor action by potentiating immunological functions of a host, by acting to suppress or reject the carcinoma in the host. This therapeutic method has been initiated by the BCG-therapy introduced by Mathé *et al.* (1969)[1] and Morton *et al.* (1974)[2]. Since then, various immunopotentiators have been developed and clinically tested in almost all countries in the world.

In the present work, the non-specific immunotherapy with the use of an immunopotentiator, OK-432, was tested for the treatment of urogenital carcinoma as the fourth therapeutic means in addition to conventional surgical operation, chemotherapy and radiation-therapy. The effectiveness of the therapy was evaluated from the observed values of various immunoparameters. The results thus obtained from 54 cases are described below.

MATERIALS AND METHODS

The cases studied consisted of 54 cases including those with urogenital carcinoma and those for the controls, i.e., 8 cases with bladder cancer, 3 cases with ureteric cancer, 9 cases with renal cell carcinoma, 2 cases with renal pelvic carcinoma, 10 cases with prostatic cancer and 6 cases including cancers of the penis, testis and uterus, remaining 16 cases consisting of controls and non-carcinomatous patients (Table 1).

Immunopotentiator (OK-432, Chugai Pharm. Co. Ltd.)

The OK-432 is a lyophilized cell preparation of the low virulent Su-strain of strep-

Table 1. Materials

Urogenital cancers and control groups ; 54 cases	
a) bladder ca.	8 cases (♂ 7, ♀ 1)
b) ureteral tumor	3 cases (♂ 2, ♀ 0)
c) renal cell ca.	9 cases (♂ 8, ♀ 1)
d) renal pelvic ca.	2 cases (♂ 2, ♀ 0)
e) prostatic ca.	10 cases (♂ 10)
f) cancers of penis, testicle, uterus and others	6 cases
g) control group and non-cancerous patient cases	16 cases

tococcus pyogenes (group A, type 3) treated with penicillin G. which is known to have anti-tumor effect. One K.E. as clinical unit of this preparation corresponds to 0.1 mg of the cell dry weight. The anti-tumor effect of OK-432 is exerted mainly by enhancing host immune responses, i.e., activation of macrophages and lymphocytes, and enhancing delayed hypersensitivity skin reactions to PPD and PHA. It has also been clinically noted that, in addition to potentiation of reticuloendothelial function, OK-432 may cause the increase of the lymphocyte numbers of peripheral blood, rising of the lymphocyte blastogenesis to PHA and Con. A.

Intracutaneous administration of OK-432

Intracutaneous injection method of the immunopotentiator OK-432 has been reported to potentiate the reticuloendothelial system and to strongly stimulate the immunoactivity, as suggested in intracutaneous vaccination of B.C.G. (Morton *et al.*, 1974).[2] Therefore, OK-432 was intracutaneously given to patients in this present work, the injection method employed being the modified intracutaneous vaccination

Table 2. Comparative results by intracutaneous and intramuscular administrations of OK-432.

cases	route	local findings					general condition			
		pain	heat sensation	absorption	scar	ulcer	fever	paresthesia	chill	anorexia
5 ※	i.c. adm.	(−)	(−)	2～3 hours	(±)	(−)	0/5 (0%)	(−)	(−)	(−)
5 ※	i.m. adm.	(±)	(+)	2～3 hours	(−)	(−)	5/5 (100%)	(+)	(+)	(±)

cases	route	before or after adm.	skin test			lymphocyte blastogenesis		leucocyte count	lymphocyte count	TB-Subpopulation		IgG−FCR+ T-cell
			PPD	PHA	Su-P	PHA (20γg)	Con-A (10γg)			T-cell	B-cell	
5 ※	i.c.	before	2.0	0.9	2.5	28,925	30,610	3,800	1,140	82	3	16
	adm.	after	2.4	1.0	2.8	28,046	32,610	4,600	1,472	82	7	12
5 ※	i.m.	before	1.7	1.1	1.8	37,601	28,074	4,000	1,200	65	6	5
	adm.	after	1.7	1.1	1.7	38,107	28,925	5,200	1,560	70	18	8

※ materials are non-cancerous patients and the period of study in 7 days

cases	route	before or after adm.	skin test			lymphocyte blastogenesis		leucocyte count	lymphocyte count	TB-Subpopulation		IgG FCR+ T-cell
			PPD	PHA	Su-P	PHA (20γg)	Con-A (10γg)			T-cell	B-cell	
ureteral tumor ♂ 63 years-old	i.c.	before	1.4	3.0	3.5	10,234	9,864	4,600	2,208	79	9	5
	adm.	after	2.0	3.5	4.3	12,652	11,583	7,300	2,482	84	10	7
prostatic ca. ♂ 76 years-old	i.m.	before	0.8	1.6	3.5	10,349	13,095	4,000	1,400	62.6	35.4	13
	adm.	after	1.2	1.7	3.5	13,871	13,961	5,800	2,773	80	31	9

method of Hoshino *et al.*[3] (1976). Thus, OK-432, 1~2 KE, suspended in 1 ml of 1% procaine solution was slowly injected intracutaneously at the brachial deltoid muscle portion using a tuberculin injector. The dose for a cancer patient was selected to be 2.0 KE which was given 3 times a week as a rule.

The immunotherapy was commenced immediately after the termination of various laboratory tests for hospitalized patients. Immunochemotherapy, surgical therapy and radiation therapy were also performed, in principle, in parallel to the immunotherapy with OK-432.

Parameters for immunoactivity

The following tests were performed in order to obtain the values of parameters which may reflect the immunoactivity of a host: the counts of leucocyte and lymphocyte in the peripheral blood, delayed hypersensitivity skin test to PPD and PHA (Welcome), and Su-polysaccharide skin test. PHA, Con. A-induced blastogenesis by means of the total blood method, countings of T. B. subpopulation and of IgG-FcR^+ T cell etc. Also, the normal values of respective immunoparameters are as follows: counts of leucocyte (average, 6500), counts of lymphocyte (average 25%, 1600~2000), delayed hypersensitivity skin test to PPD (10 mm or over/48 hrs), to PHA (10 mm or over/24 hrs), Su-polysaccharide test (10 mm or over/24 hrs), induced blastogenesis of lymphocyte (S.I. or c.p.m.), T cell (70–85%), B cell (5–20%), IgG-FcR^+ T cell (2–8%). These valus were measured before and after the administration of OK-432. The side action of OK-432 was also examined.

As intramuscular injection is widely employed for the administration of OK-432, and the occurrence of side effects due to intramuscular administration including pyrexia was compared with that due to the intracutaneous injection which was used in the present work.

Cases studied were classified into the following groups: Group A: non-cancer patients, Group B: only surgical operation for cancer patients, Group C: cancer patients treated with chemotherapy, surgical operation and OK-432, Group D: cancer patients treated with multiple chemotherapies, surgical operation and OK-432, Group E: prostatic cancer patients surgically treated with castration and treated with anti-androgenic hormone and OK-432, and Group F: patients treated with OK-432 after the remission of the carcinoma. Two additional groups of non-cancer patients were also provided for the comparison of side effects of OK-432 due to intramuscular and intracutaneous injections. Representative cases were picked up from these patient groups taking the age factor into account and limiting the severity to the range of T_2-T_3, and variations of each of the parameters were graphically presented (Table 2).

RESULTS

Comparison of intramuscular injection with intracutaneous injection of OK-432 (Table 3)

The administration of OK-432 is usually carried out by means of intramuscular injection and it is known to result in unpleasant pyrexia as a side effect of this substance. As shown in Table 3, the results obtained with non-cancer patients indicated that pyrexia and local pain were not induced by intracutaneous injection of OK-432. In addition, no formation of an ulcer was demonstrated at the locus of the injection. It was also shown that OK-432 was absorbed within 2~3 hrs into the body. The immuno-parameters were not affected, of course, by either the intracutaneous or intramuscular injection of OK-432 into non-cancer patients, while the intracutaneous injection of OK-432 into cancer patients stimulated the reticuloendothelial system, thus enhanced the immunoactivity of the host, in a manner similar to that due to intramuscular injection of OK-432. Therefore, it may be concluded that intracutaneous injection of OK-432 is superior to conventional intramuscular injection on the basis of the lack of any apparent side effect in intracutaneous injection of this substance.

Table 3. Classification of groups and their representative cases.

classification	group	classification	representative cases								
			classification	classification	diagnosis	T-classification	metastasis	therapeutic method			
								surgical operation	chemotherapy	radiotherapy	immunotherapy
A	non-cancerous patients group	6	78	♂	B.P.H			prostatectomy			
B	cancer op. group	4	29	♂	testicular tumor	T_2	(−)	orchiectomy	(−)	(−)	(−)
C	cancer patients chemotherapy+op +OK-432, group	11	61	♂	bladder tumor	$T_1 \sim T_2$	(−)	T.U.R.Bt	Bleomycin	(−)	OK-432
D	cancer patients multi-drug combination+op+OK-432, group	6	56	♂	renal cell ca.	T_3	(−)	nephrectomy	FAMT Adriacin	(−)	OK-432
E	prostatic cancer group castration+antiadrogenic therapy +OK-432, group	10	66	♂	prostatic cancer	T_3	(−)	castration	(−) antiandrogenic therapy (+)	(−)	OK-432
F	OK-432 administered group after remission of cancer	7	48	♂	ureteral tumor	T_2	(−)	nephro-ureterectomy	(−)	(−)	OK-432

controls (normal healthys) 10 cases, 54 cases in total

Therapeutic effect of OK-432 for cancer patients

Various immunoparameters were obtained before and after the commencement of the therapy at two-week-intervals from all the patients investigated. The comparison of variations of the parameters were carried out with representative cases selected from each of the groups.

a) The counts of leucocyte and lymphocyte in peripheral blood

The counts of both leucocyte and lymphocyte were found to increase or to be around normal levels in all cases treated with OK-432, whereas the group D which was treated with multiple chemotherapeutic drugs demonstrated decreased levels of these cells, and groups A and B to which OK-432 was not administered also showed low levels of both leucocytes and lymphocytes (Fig. 1).

b) Skin tests

Results of PPD skin test (delayed hyper-

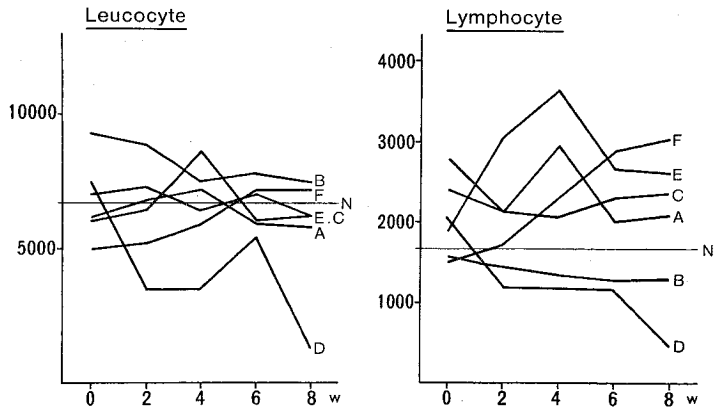

Fig. 1. Variation of leucocytes and lymphocytes on pre-post therapy with OK-432.

sensitivity reaction) showed low values for the group A. This might be due to the age factor for this group. Other group showed higher values except for the group D which exhibited the declining curve.

In PHA skin test, abnormally high values for the group A and declining curves for the group D were most distinguished. No correlation was observed between the results of PPD and PHA skin tests.

As Su-polysaccharide test is specific to OK-432, all cases treated with OK-432 showed positive reaction (Fig. 2).

c) Induced blastogenesis of lymphocyte (PHA, Con .A)

Although the induced blastogenesis of lymphocyte is usually examined by measuring the responses to PHA and Con.A, it is suggested by other investigators that these values vary considerably and that it is often improper to express the blastogenesis in terms of the stimulation index (S. I.) obtained from these values. In order to avoid such uncertainty, the graph was drawn at levels of 20/μg PHA (c.p.m.) and 10/μg Con.A (c.p.m.). The results thus obtained showed a common pattern in curves for the PHA and Con.A. Groups treated with OK-432 showed increasing patterns, while the group D exhibited decreasing curves. The decrease observed in the group A might be attributed to the influence from surgical operation and that from the age of the patients.

A correlation was suggested between the results of PHA skin test and the induced blastogenesis of lymphocyte obtained by PHA measurement (Fig. 3).

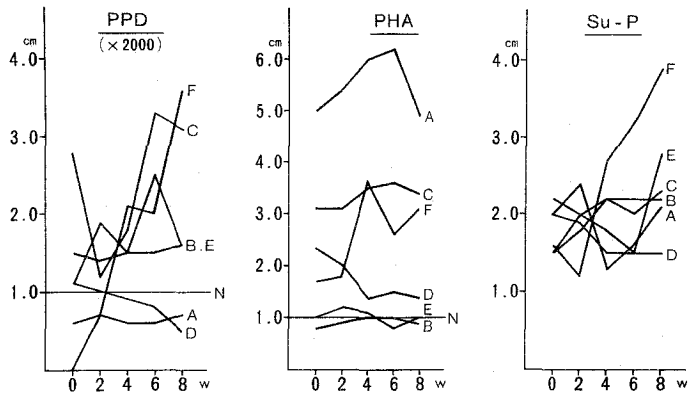

Fig. 2. Variation of PPD skin test and PHA skin test and Su-polysaccharide test on pre-post therapy with OK-432.

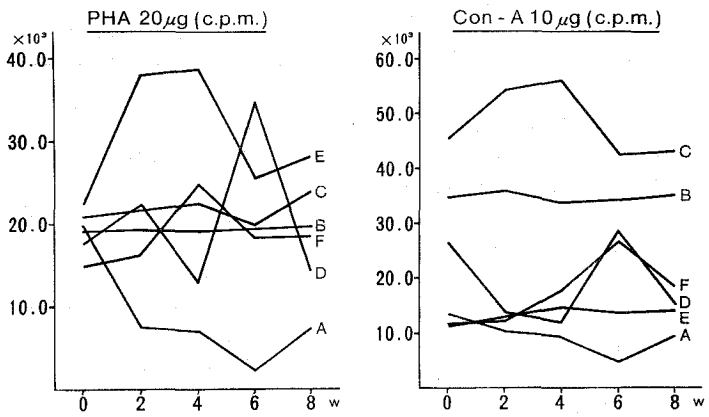

Fig. 3. Variation of lymphocyte blastogenesis (PHA, Con-A) on pre-post therapy with OK-432.

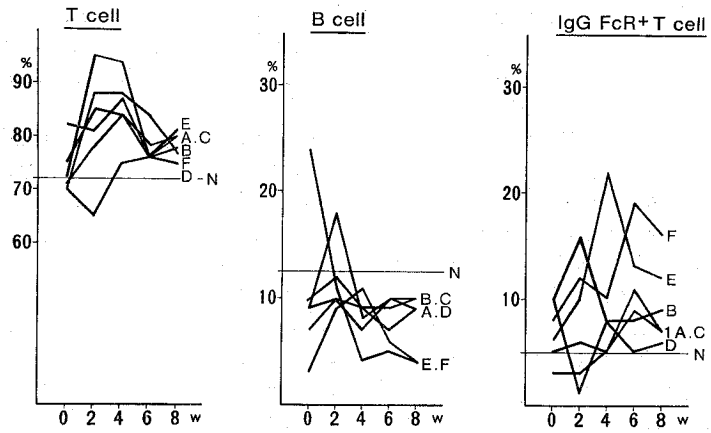

Fig. 4. Variation of T.B. subpopulations (T cell, B cell, IgG FcR+ T cell) on pre-post therapy with OK-432.

d) T. B. subpopulation

It was found that T. B. subpopulation was low in cases of highly advanced carcinoma (metastasis cases) which belonged to the grade T_3 or T_4 according to the T-classification. It was also observed, however, that the case which had shown a low T cell level improved or returned to the normal level by the treatment with OK-432, and the case which had an abnormally high level also approached to the normal level in the course of the therapy. The group D did not give the curve lower than the normal value.

Level of B cell were generally lower than the normal level in the patients examined and thus seemed to be in a relation opposite to the T cell. Although there were two groups before the therapy, the one with high B cell level and the another with low B cell level, there was a tendency that these B cell levels approached to the normal level in association with the restoration of the T cell level during the therapy with OK-432.

IgG-FcR+ T cell is known to give a pattern significant in reflecting the host immune response. It was demonstrated that the administration of OK-432 clearly restored the reactivity of the host according to the curves obtained from IgG-FcR+ T cell. The curves for groups A and B which were not given OK-432, however, were at lower levels than those of other groups; this might be due to factors of the age and surgical operation. The administration of OK-432 certainly restored the level of IgG-FcR+ T cell, the subset of T cell, after the performance of surgical operation or chemotherapeutic treatment, as indicated by the increasing curves given (Fig. 4).

According to the results obtained in this study, it may be concluded that the non-specific immunotherapy with intracutaneous administration of OK-432 is relatively easy in application, only with minor side effects and might be effective in preventing, to some extent, the side effects due to the chemotherapy, such as leukopenia and depilation. It was also demonstrated in the present work that the therapy with OK-432 given by intracutaneous injection method may enhance the immunoactivity of the host, thus leading to the prolonged life of the host.

It was shown that the immunoactivity of the cancer-bearing host may well reflect the host immunoactivity, which is sensitive to drastic surgical operation procedures, radiation-therapy, chemotherapy with the anti-cancer agent and the age factor. In the case of multiple administrations of large amounts of anti-cancer drugs, however, the lowering of the host immunoactivity seems not to be completely prevented by OK-432 (Table 4).

According to the immunoparameters measured in this work, levels of T cell and mitogen (values of PHA and Con.A) were found to become lower with the progression

Table 4. General results.

group	cases	T classification				variation of calculated activities for immunological parameters									side effects and others				
						skin test			lymphocyte blastogenesis		T.B. subpopulation			leucocyte	lymphocyte	baldness	leukopenia	anorexia	nausea	fever
		T_1	T_2	T_3	T_4	PPD	PHA	Su-P	PHA	Con-A	T cell	B cell	IgG FcR^+							
A	6	0	0	0	0	(+)	(+)	(+)	normal or lower after operation	normal or lower after operation	normal	normal	normal	normal	normal	—	—	—	—	—
B	4	1	2	1	0	(+) ↓ (−)	(+)	(+)	lower after operation or normal					normal	normal	—	—	—	—	—
C	11	2	6	3	0	(−) ↓ (+)	(−) ↓ (+)	(+)	elevated	elevated	increased	increased	increased	normal	normal	—	—	—	—	—
D	6	0	1	4	0	became negative during chemotherapy and positive subsequently		(+)	lower during chemotherapy and recovered subsequently					decreased	decreased	+	++	++	+	±
E	10	0	3	6	1	(−) ↓ (+)	(−) ↓ (+)	(+)	elevated	elevated	normal increased	normal increased	normal increased	increased	increased	—	—	—	—	—
F	7	0	5	2	0	(−) ↓ (+)	(−) ↓ (+)	(+)	elevated	elevated	normal increased	normal increased	normal increased	increased	increased	—	—	—	—	—

of the carcinoma. The level of IgG-FcR^+ T cell also decreased with the severity of the carcinoma and was also affected by surgical procedures and the age factor. The administration of OK-432 restored the reactivity of the T cell and consequently improved systemic conditions of the patients.

DISCUSSION

The immunotherapy for carcinoma has recently become one of therapeutic methods which are attracting world-wide attention. This method, however, is basically the non-specific immunotherapeutic means, thus it is to be employed supplementally to surgical operation, radiation-therapy or chemotherapy[4].

The immunotherapy for cancer which is non-specific in nature, has been initiated by the application of B.C.G. to the leukemia by Mathé *et al.* (1969)[1] and to the melanoma by Morton *et al.* (1972)[2], and since then numbers of immunopotentiators have been developed and clinically tested.

It has been pointed out by Kimura *et al.*[4], as to clinical application of immunochemotherapy, that the efficacy of the non-specific immunotherapeutics to the carcinoma naturally has a certain limitation when the agent is used singly. The activity of such drugs is usually slow-developing and is largely influenced by the number, size, developmental stage and extent of the spread, of a cancer cell at the stage of therapeutic treatment. Therefore, the agent should be used as one of immunochemotherapeutic means in order to obtain the highest effectiveness.

The immunotherapeutics seem to prevent development of side effects of chemotherapeutics, such as the decrease of leucocyte numbers, this being one of the merits of this method. As the immunotherapy is thus supplemental to the chemotherapy as to the side effect and the influence to the host, it is convenient to clinically apply the immunotherapy as a supplemental therapeutic means.

OK-432 has been widely used clinically as the immunotherapeutics for the carcinoma in association with the chemotherapeutics resulting in respective curative

effect[4~8]. This substance was first discovered, in the hemolytic streptococci, to have enhancing effect on host immune response and also to have the activating effect on macrophages and lymphocytes.

In order to evaluate the effectiveness of the immunotherapeutics with certainty, the examination should be carried out with the cases which are therapeutically treated on a common basis and by the randomized trial method, and it is also necessary to observe various immunological parameters. Clinically speaking, however, the parameter which may most faithfully indicate the immuno-condition of a patient and may give information as to therapeutic effect and the post-treatment condition is not defined yet[9].

In the present study, the parameters which have been most widely and most frequently employed and investigated, such as the induced blastogenesis of lymphocyte (PHA, Con. A), T. B. subpopulation, the delayed hypersensitivity skin reaction (P PD), PHA skin test and the leucocyte count in peripheral blood and lymphocyte count were tentatively examined. Yata *et al.*[10] has reported that the measurement of IgG-FcR$^+$ T cell level in association with that of T. B. subpopulation may well reflect the host reactivity as well as the observation on mitogens. We also agree with his remark. These parameters are important in indicating the relation between in vitro parameters and the extent of progression of the disease or post-treatment condition of a patient. The induced blastogenesis of lymphocyte, T. B. subpopulation and IgG-FcR$^+$ T cell are also considered to be suitable as a means to indicate a non-specific cellular immunity. From the point of view against cancer immunity, it is considered that the cellular immunoactivity mainly consisted of the function of the T cell is important as a diagnostic parameter for the non-specific anti-cancer immunotherapy.

The intracutaneous injection method of the immunopotentiator OK-432 into patients with urogenital carcinoma was performed, as the immunochemotherapy, in the present work; however, there seemed to be no dose-response relation. Side effects of the chemotherapeutics used as an immuno-suppressor were possibly prevented by the co-administration of OK-432[11].

Since the immunoactivity of the host has changed by the administration of OK-432, it is a drug to be clinically tried because of possibility to prolong the host's life.

In view of the present progress in clinical application of the non-specific immunotherapy throughout the world, the establishment of the specific immunotherapy should be expected in the future.

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和文抄録

尿路性器癌に対する Immunopotentiator OK-432 による免疫療法

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尿路性器癌に対する治療法として非特異的免疫療法は，今日，外科的療法，化学療法，放射線療法に加えて非常に脚光を浴びている．われわれは，尿路性器癌54例に対し immunopotentiator OK-432 を皮内投与し，その担癌宿主の免疫賦活作用を検討した．

検討項目は，末梢血の白血球数，リンパ球数，遅延型皮膚反応として PPD skin test および PHA skin test，mitogen PHA，Con-A によるリンパ球幼若化率，T，B 細胞測定および IgG-FcR$^+$ T cell の測定を施行した．結果として，OK-432 の皮内投与法は容易に施行でき，発熱などの副作用もなく，抗癌剤などの投与時に起こる白血球数減少もある程度防止できることを知った．

そして，その細胞性免疫能は OK-432 の投与により回復することが認められ，宿主の延命効果に役立っているように思われた．

訂正：Table 4. Group B の PPD 欄の $\frac{1}{4}$ は $\frac{1}{4}$ の誤りです．