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Study on Immunosuppressive Substance in Gastric Cancer

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ABSTRACT : The serum immunosuppressive (IS) levels were measured in the 73 gastric cancer patients and analysed in terms of histoclinical factors of the progression of carcinoma. And also in vitro study was made to clarify the action of IS substrance on lymphocyte blastogenesis, NK and LAK cell activities.

1) The serum IS levels in gastric cancer patients increased in accordance with progression of carcinomas. It is of great benefit to assess the advancing cancer progression and to know the prognosis.

2) The serum IS levels inversely proportionated to lymphocyte blastogenesis, NK and LAK cell activities, in particular, showed close correlation with LAK cell activity.

3) In vitro study clearly indicated that IS substance inhibited lymphocyte blastogenesis, as well as NK and LAK cell activities in accordance with IS dosis-dependent manner.

INTRODUCTION

Recent study clarified a presence of immunosuppressive substance (IS) in the sera of tumor-bearing hosts.

Fuji reported a presence of IS, glycoprotein, molecular weight 52,000, in the sera of gastric cancer patients, many investigators reported that IS could be not infrequently found in the sera of healthy humans but it increased in tumor-bearing hosts in proportion to immunodepressive states.

However, the mechanism to depress the immune states was not entirely clarified to date. The purpose of this study is to clarify the correlation with histoclinical factors of gastric cancers and the influence on lymphoblastogenesis, and NK and LAK activities.

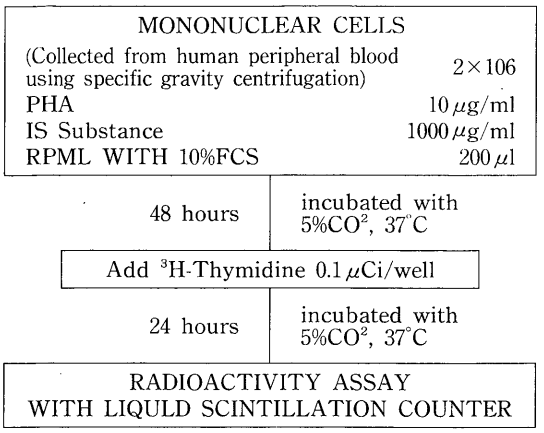
MATERIAL AND METHOD

IS measurement was performed in 73 gastric cancer patients in contrast with the control in whom included 20 benign diseases and 15 healthy persons. Gastic cancer patients comprised of 42 men to 31 women and ranged from 33 to 75 years old (with an average of 57.9±9.9). On the other hand, benign disease patients were composed of 8 gastric ulcer, 12 cholecyto cholelithiases, with the ratio of 14 men to 6 women, ranging from 40 to 72 years old with an average of 57.3±9.7 and healthy candidates were 10 men and 5 women, ranging from 29 to 68 years old, averaged 46.3±10.7 years old.

IS measurement was performed according to single radical immunodiffusion method using anti-IS rabbit serum plates.

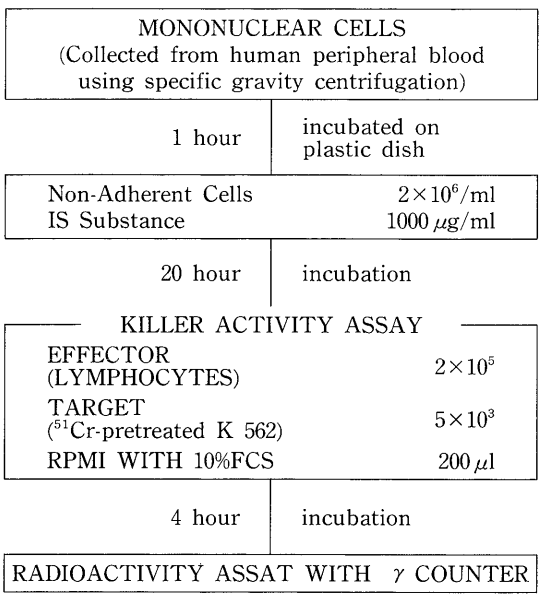
Blastogenesis was measured in accordance with the steps as described in **Fig. 1.**

The peripheral blood was collected with heparin using specific gravity centrifugation with the use of Ficoll-Hypaque solution. Adding 10 g/ml PHA at 67°C, a 48 hour culture was achieved in addition to 0.1 Ci/well ^{3}H-thymidin, washed and countered using by Liquid Scintillation Counter to get an intake of thymidine content as an expression of stimulation



STIMULATION INDEX (S. I.)=
$$\frac{\text{cpm SAMPLE with PHA STIMULATION}}{\text{cpm SAMPLE without PHA}}$$

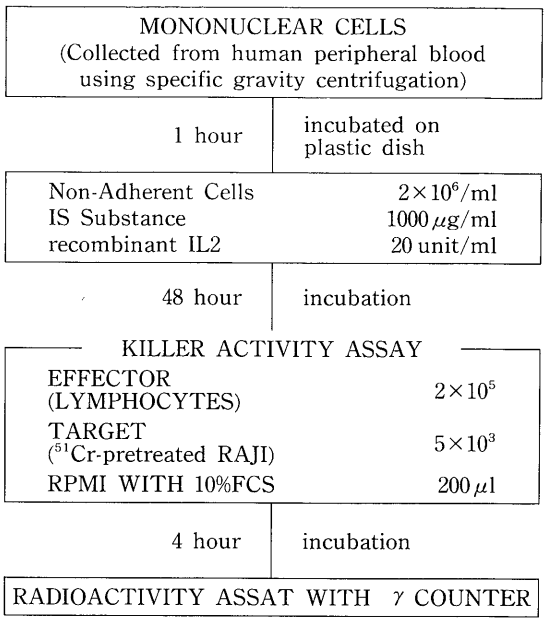
Fig. 1. The shema of the assay for T cell proliferative response



KILLER ACTIVITY=%SPECIFIC LYSIS=$\frac{E-S}{M-S}$

E: EXPERIMENTAL RELEASE
M: MAXIMUM RELEASE WITH IN HCI
S: SPONTANEOUS RELEASE

Fig. 2. The schema of the assay for natural killer activity



KILLER ACTIVITY=%SPECIFIC LYSIS=$\frac{E-S}{M-S}$

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M: MAXIMUM RELEASE WITH IN HCI
S: SPONTANEOUS RELEASE

Fig. 3. The shema od the assay for lymphokine activated killer activity

index (SI) as shown in **Fig. 1.** For in vitro study, pure substance supplied from Kureha Co was prepared. NK activity was measured as summarized in **Fig. 1.**

Human peripheral blood was collected with heparin and monocytes were provided using specific gravity centrifugation and cultured for 1 hour in plastic dishes, non-adherent cells were collected in 10%. FCS+RPMI 1640 and prepared as 2×10^6 monocytes in suspension. K562 cells labelled with ^{51}Cr were prepared for a target cell. Admixture of a ratio of 40:1 of E:T was cultured for 4 hours and the radioactivity assay of the surpernatant was made by using r-counter. Radioactivity was expressed as % specific lysis as shown in **Fig. 2.**

In vitro study 1000 g/ml purified IS was added to culture medium and incubated for 20 hours on various conditions.

LAK activity test was performed as summarized in **Fig. 3.** Non-adherent cells in plastic

dishes as described in measuring NK activity incubated in 5% CO_2 at 37°C for 48 hours in admixture with 20μ/ml recombinant interleukin 2 (Ruk-2). Taget cell was labelled with ^{51}Cr in admixture with a ratio of E : T of 40 : 1. The action of 15 was tested by the addition of 500g/ml pure IS in vitro study. The values of mean and standard errors were statistically analysed by student test.

RESULTS

Serum IS concentrations averaged 661±230g/ml in gastric cancer patients, In contrast, those in healthy subjects were 507±104g/ml and in benign diseases, 527±119g/m. With advances in disease stage, those were 541 ±118g/ml in stage I, 618±267g/ml in stage II, 696±237g/ml in stage III and 898±413g/ml in stage IV. Serum IS apparently increased in stage IV ($p<0.01$). Positive rates in which the value of 700mg/ml were regarded as 100, were 5.9% in healthy subjects, 9.1% in benign deseases, 10.3% in stage I 25.0% in stage II 52.4% in stage III and 66.7% in stage IV. IS level over 1000 g/ml included all 11 patients with advanced cancer, in particular 73% out of the 11 patients corresponded to stage IV gastric cancers (**Fig. 4**).

According to lymphnode involvement, the IS levels were 545±117 g/ml in n_0, 629±262 in n_1 876±438 g/ml in n_2, 843±351 g/ml in n_3 and positive rates were 12%, 30%, 69%, 58% respectively (**Fig. 5**). The IS levels were temporarily increased immediately after surgery and 3 weeks later, these fell down below the prior level in curative operation. On the other hand, it took about 3 months after surgery until reached to previous level in patients with non-curative operation. In contrast, these remained still high in patients with not resected surgery (**Fig. 6**). **Fig. 7** showed close correlation with serum IS level and CD4/CD8. The higher the serum IS levels, the lower CD4/CD8

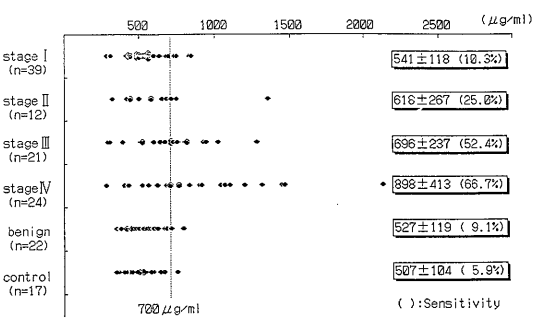

Fig. 4. The serum IS titer in patient with gastric cancer according to clinacal stage

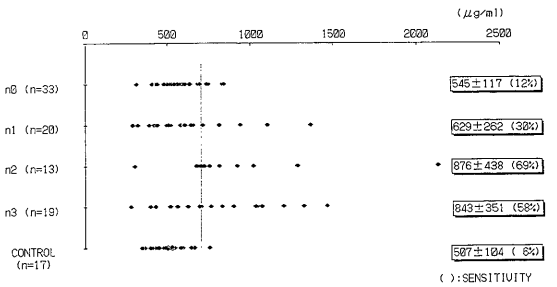

Fig. 5. The serum IS titer in patient with gastric cancer according to lymph node metastasis

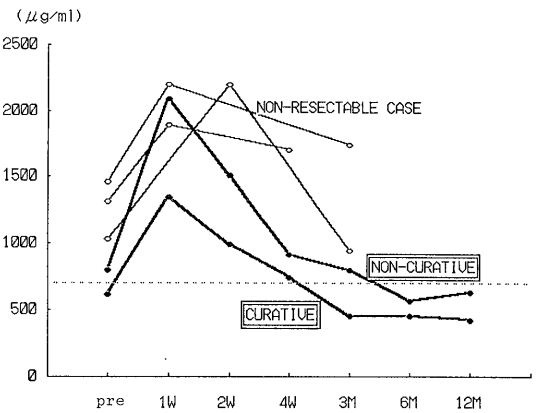

Fig. 6. The changes of serum IS titer after operation. Means of IS titer of resectable cases (closed circle) and individual IS titer of non-resectable case (open circle).

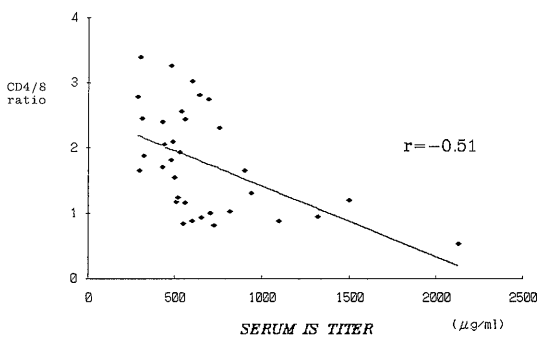

Fig. 7. The influence of serum IS titer on CD4/8 ratio of lymphocyte subpopullation

had shown.

In comparison with the serum IS levels and T cell proliferative responses there was inverse proportion indicating the coeficient index of 0.46 as shown in **Fig. 8.**

On the other hand, NK and LAK activities were compared with serum IS levels. An increase in serum IS levels had led to reduced NK and LAK activities. The coeficient indices were −0.54 in NK activity and −0.62 in LAK activity as shown in **Fig. 9** and **10.**

Fig. 11 showed the effect of the addition of purified IS on T cell proliferative response.

In addition of 500 g/ml and 1000 g/ml purified IS, **Fig. 11** showed that SI were depressed to 87.0±30.3 and 70.0±28.1 g/ml, indicating a dosis-dependent relationship as compared with 97.6 ± 32.9 without purified IS **Fig. 12** showed that the addition of 1000 g/ml purified IS during a 20 hour incubation time of NK activity measurement allowed a slight depression of 51± 13.4 as compared with 56.4±13.1 of no addition of purified IS ($p<0.05$).

LAK activities showed 41.6±9.6 by adding 500 g/ml IS and 28.7±7.8 by adding 1000g/ml, indicating the dosis-dependent inhibition of IS. As a result, the inhibitory rates were 83.3% by

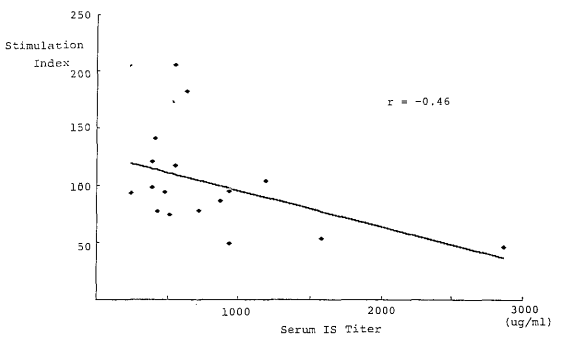

Fig. 8. The influence of serum IS titer on T cell proliferative response

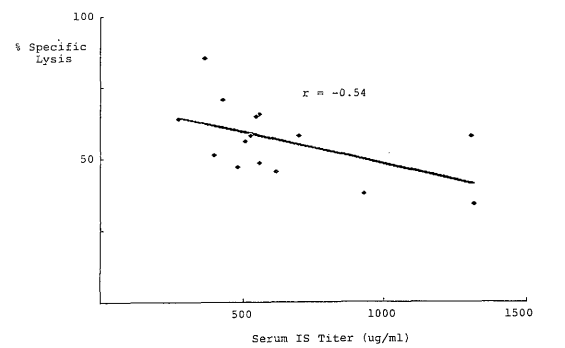

Fig. 9. The influence of serum IS titer on natural killer activity

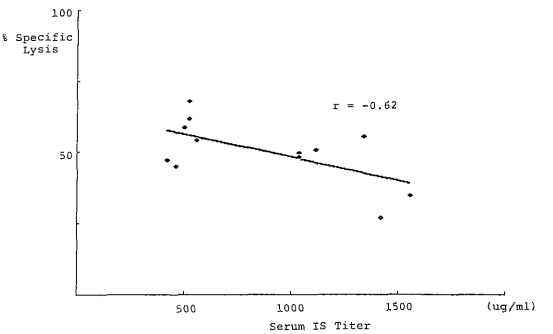

Fig. 10. The influence of serum IS titer on lymphokine activated killer activity

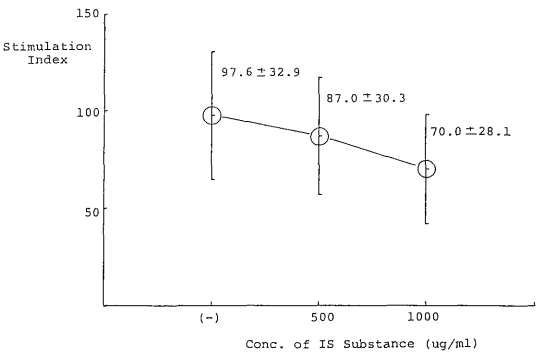

Fig. 11. The effect of IS substance on T cell proliferative response. IS sibstance was added into medium during culture with PHA.

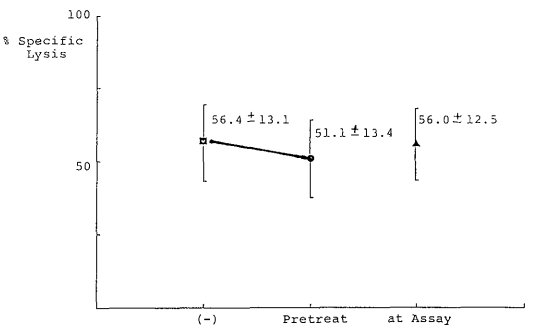

Fig. 12. The effect of IS substance on natural killer activity. IS substance (1,000 ug/ml) was added into medium 20 hours before (Pretreat) and during (at assay) cytotoxic assay.

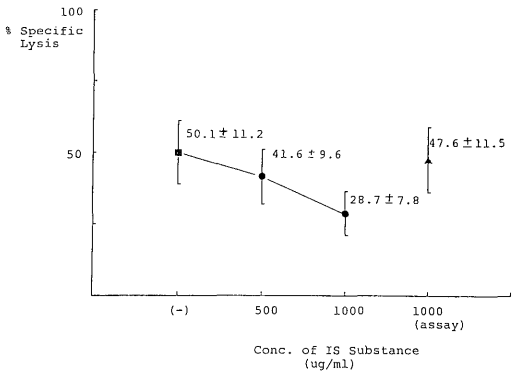

Fig. 13. The effect of IS substance on lymphokine activated killer activity. IS substance (500 or 1,000 ug/ml) was added into medium before and during cytotoxic assay.

adding 500g/ml IS and 57.3% by adding 1000g/ml IS.

DISCUSSION

Many reports infer a depressed immune response in tumor bearing hosts, However, the mechanism is not yet obvious. It is widely accepted that one of them is associated with a presence of the immunosuppressive substance which was named immanosuppressive acidic protein (IAP) by Matsuda[8] and IS by Fujii[2].

There are reports that IAP and IS levels were reduced in the serum of a tumor-bearing host, indicating close relation to inhibited immune response.

A significant increase in serum IS as the tumor marker was reported in gastric cancer, colon cancer and carcinoma of the bile duct by Manami. Matsumoto[5] clarified that IS levels indicated progression of the tumors in colon cancer. Noguchi[4] also reported that an increase in the serum IS was remarkable in advanced and recurrent uterine cancers.

In this series, with advances in the disease stages the serum IS levels rose up. However positive rates were still lower even in early gastric cancer. It reflected that serum IS levels were inadequate for tumor markers to know a presence of gastric cancer in early stage.

It is substantiated that changes in serum IS levels closely refect the prognosis and it is of great benefit to know recurrence in early stage.

To elucidate the immune state of tumor-bearing hosts, delayed reaction of PPD used to be clinically available for clinical assessment. In this study it was certained that serum IS levels were in close proportion to CD4/CD8, NK and LAK activity and it is suggestion that serum IS levels should be an expression of the immune response of the tumor-bearing host.

It is well known that T cell proliferative response is influenced by the action of interleukin 2, macrophage and serum immunosuppressive substance, which is generated by peripheral lymphocytes, subsequently acts on peripheral lymphocytes and exhibits the immunosuppressive effects. It is elucidated in this study that lymphoblastogenesis stimulated by PHA was inhibited by the addition of purified IS in culture medium in accordance with dosis-dependent order.

The cytotoxic test by using NK and LAK cells is now ongoing in addition of various kinds of cytokines. In this study it is clarified that cytotoxic effects of NK and LAK cells are affected by the time of adding IS substance prior to or immediatly before cytotoxic assay. Furthermore, the addition of IS substance just before cytotoxic assay does not demonstrate the inhibitory effect on cytotoxity with an aid of NK and LAK cells. From the standpoint of the result of this study, it is emphasized to assume that the inhibitory effect of IS substance on LAK cells is based on the inhibited response to LAK cells.

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