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Pregnancy-Associated Glycoprotein (PAG) in Patients with Cancers

— Synthesis from Leukocytes and Presence in the Pathologic Mucous Membrane of Colon —

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SUMMARY

PAG was synthesized from both lymphocytes and granulocytes taken from the peripheral bloods of normal subjects and from the joint fluids of rheumatoid patients as well.

PAG was found in the epithelial cells of mucous membrane of colon obtained from patients suffering from drug-induced colitis.

Key words: Pregnancy-associated glycoprotein (PAG), Leucocyte, Colon, Cancer

INTRODUCTION

The PAG levels in sera of cancer patients were compared with the serum levels of CEA or AFP and further with cellular immunity of the patients tested by PHA response of their lymphocytes.

Production of PAG from the leukocytes was attempted in culturing the cells with estrogen and the PAG present on the cells and secreted in the medium was measured by ^{125}I-labeled anti-PAG antibody.

A presence of PAG in the normal and pathologic tissues was surveyed by means of the indirect immunoperoxidase method.

MATERIALS AND METHODS

Quantitative estimation of PAG: Single radial immunodiffusion method was used to measure PAG amounts in using a pooled pregnancy serum as a standard which has 100 units of PAG.

Lymphocytes and Granulocytes: Lymphocytes were prepared from the heparinized blood through the specific gravity centrifugation in using Lymphoprep (Daiichi Chemical, Tokyo) and granulocytes were obtained from the most bottom layer of

the Lymphoprep preparation in eliminating the red blood cells by hypotonic lysis.

Tissues: The tissues of gastric cancer and colon cancer were obtained by surgical operation. The tissues of colon of patients with ulcerative colitis and drug-induced colitis were obtained by biopsy.

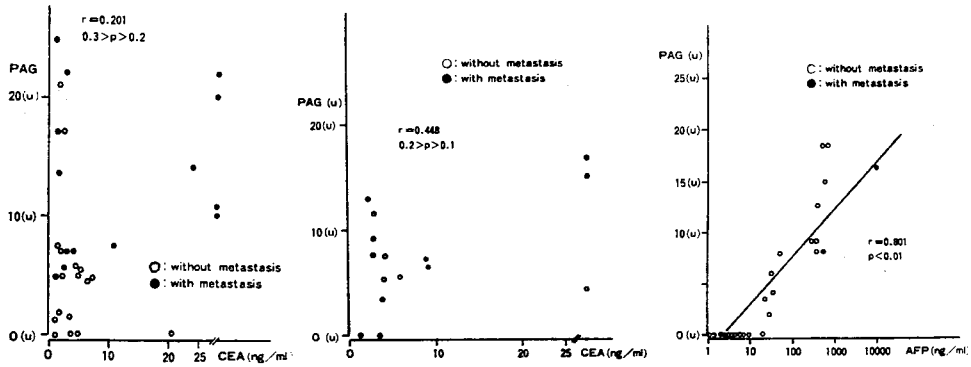
Lymphocyte response to PHA: 1×10^6 lymphocytes in the medium RPMI 1640 were mixed with 15 μg of PHA-P (Difco. Detroit) and cultured for 72 h. The incorporation of ^{3}H-thymidine into lymphocytes was measured. The stimulation index (S. I.) was expressed by a ratio of radioactivity of PHA-stimulated to unstimulated lymphocytes.

Synthesis of PAG from lymphocytes and granulocytes: One half ml of lymphocytes or granulocytes at a concentration of 4×10^6/m*l* was mixed with 0.1 m*l* of estrogen at various concentrations ranging from 0.04 to 4000 μg/m*l*. The mixture was cultured for 7 days. Obtained culture medium and the 3 times washed cells were tested for the presence of PAG by a solid-phase radioimmunoassay and a membrane radioimmunoassay using ^{125}I-anti PAG antibody respectively.

Detection of PAG in tissue sections: The tissue sections were made from the formalin-fixed and paraffin-embedded tissues. The tissues were deparaffinized and treated with rabbit anti-PAG antibody and then with the peroxidaselabeled goat antibody to rabbit IgG.

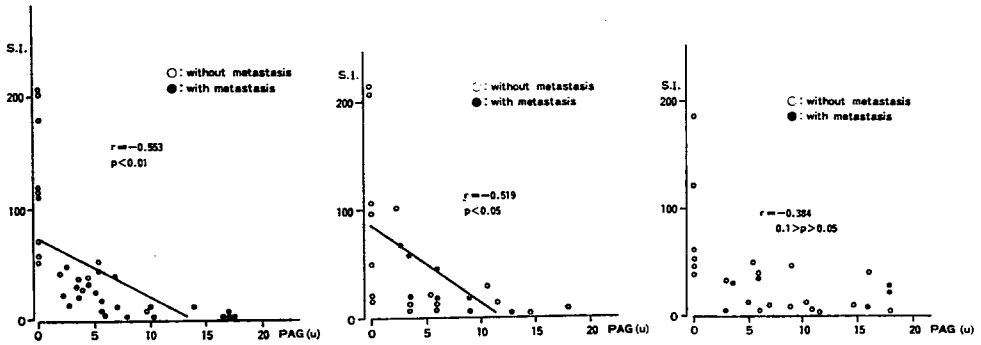
RESULTS

Higher levels of serum PAG were found in patients with various kinds of cancers, especially those with metastases.



a. gastric cancer b. lung cancer c. hepatoma

Fig. 1 Correlation between serum PAG levels and plasma CEA levels in patients with gastric cancer (in a) and lung cancer (in b). Correlation between serum PAG levels and serum AFP levels in patients with hepatoma (in c).



a. gastric cancer b. lung cancer c. hepatoma

Fig. 2 Correlation between serum PAG levels and PHA response of lymphocytes of patients with gastric cancer (in a), lung cancer (in b) and hepatoma (in c).

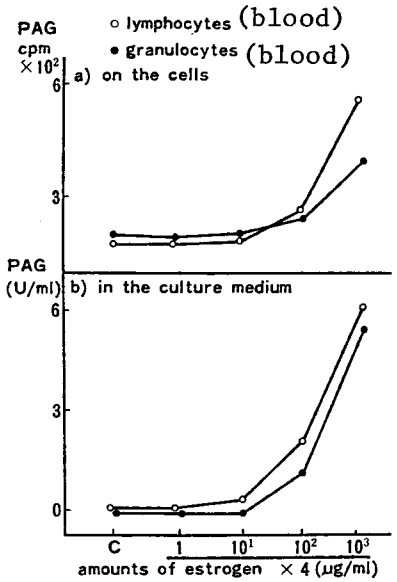


Fig. 3 Synthesis of PAG from lymphocytes and granulocytes taken from peripheral bloods of normal subjects under culture with various amounts of estrogen detected on the cell surface (in a) and in the culture medium.

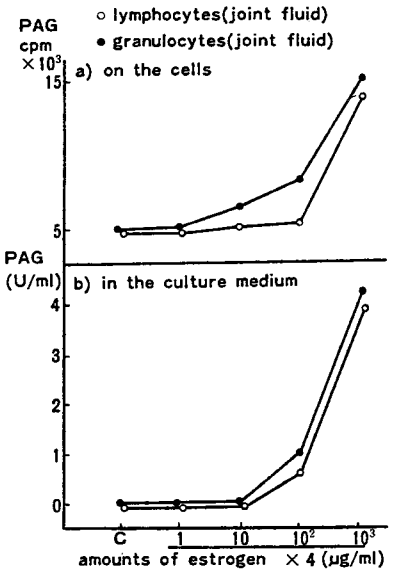


Fig. 4 Synthesis of PAG from lymphocytes and granulocytes taken from joint fluids of patients with rheumatoid arthritis under culture with various amounts of estrogen detected on the cell surface (in a) and in the culture medium (in b).

The serum levels of PAG were compared with the serum levels of other cancer-associated proteins, CEA and AFP. As seen in Fig. 1, no correlation was observed between PAG and CEA levels in patients with gastric and lung cancer (in a & b) whereas a significant correlation ($r=0.801$, $p<0.01$) was observed between PAG and AFP levels in patients with hepatoma (in c).

The stimulation index of the peripheral lymphocytes to PHA in cancer patients was compared with the serum PAG levels of these patients. As seen in Fig. 2, a significant converse correlation was observed between levels of S. I. and PAG in patients with gastric cancer ($r=-0.553$, $p<0.01$ in a) and lung cancer ($r=-0.519$, $p<0.05$ in b) but not in patients with hepatoma (in c).

The PAG was synthesized from lymphocytes and granulocytes in the peripheral bloods of normal subjects under culture with estrogen at concentrations of 400 μg/m*l* and 4000 μg/m*l*. As seen in Fig. 3, cell surface PAG (in a) and secreted PAG (in b) were observed in both lymphocytes and granulocytes. The PAG was also synthesized from both lymphocytes and granulocytes taken from the joint fluids of patients with rheumatoid arthritis similarly detected on the cell surface (Fig. 4a) and in the culture medium (Fig. 4b).

In the preliminary results, PAG was detected in the epithelial cells of mucous membrane of colon taken by biopsy from patients suffering from Penicillin-induced colitis. This type of positive findings of PAG in colon were found in 3 out of 5 patients with drug-induced colitis but in none of 5 colon cancer and 3 ulcerative colitis.

DISCUSSION

In the previous study(1), the purified PAG showed a significant suppression on the PHA response of lymphocytes. Therefore, the results observed in this study that the PHA response of lymphocytes correlated well with serum PAG levels in patients with gastric and lung cancer might accord with the previous observation. It is obscure, however, why such correlation was not found in patients with hepatoma.

The observed results that lymphocytes and granulocytes taken from the joint fluids of rheumatoid patients synthesized PAG similarly to the lymphocytes and granulocytes in the blood seemed to agree with the results observed by other investigators(2). The production of PAG from the leukocytes in the joint fluids may explain the increased levels of PAG in joint fluids of rheumatoid patients.

The definite presence of PAG in the epithelial cells of colonic mucosa of patients with drug-induced colitis may suggest a local production of PAG in pathologic tissues.

REFERENCES

1. KASUKAWA, R., OHARA, M., YOSHIDA, H. and YOSHIDA, T.: Pregnancy-associated α_2-glycoprotein in rheumatoid arthritis. Int. Arch. Allergy Appl. Immunol. **58,** 67-74 (1979).
2. STIMSON, W. H. and BLACKSTOCK, J. C.: Synthesis of a pregnancy-associated α_2-macroglobulin by human leukocytes. Experimentia **31** 371-373 (1975).