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Humoral Regulation in Cell-mediated Immunity of Cancer-patients

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INTRODUCTION

It is now well established that immune responses may be categorized by their dependence upon two functionally different cells which participate in the immune response.

The T-lympocytes play an important role in the cell mediated immunity. Sensitized lymphocytes to a target cell kill these cells when cultured together. Non-sensitized lymphocytes are cytotoxic to the target cells only when cultured with phytohemagglutinin. This phenomenon is interpreted as an immunological reaction (1, 2).

Recent evidence is accumulating, suggesting the existence of tumor specific antigens in human tumors and lymphocyte blastogenic response to autochthonous tumor cells can be detected in at least two-thirds of patients with solid tumors in the absence of any significant response to normal cells (3, 4). If tumors are antigenic and the host is capable of reacting to these antigens, how can a tumor develop and grow in the face of a potentially cytocidale immune response?

ESCAPE MECHANISM FOR TUMORS

Some mechanisms by which the host's reaction may be disarmed, are shown in Fig. 1. First, the inactivation of specific cell-mediated responses in patients in whom the tumor continues to grow, could be explained by the existence of humoral factors in the serum and extracellular fluid which are capable of interfering in some way with shedding, masking, blocking and inhibition of the immune response. Second, a general immunological unresponsiveness is demonstrated in cancer-patients. Any immunological deficit would be either the cause or the consequence of the escaping tumor. Third, the function of T-lymphocytes which is the initiation of antibody responses is well established and it is becoming clear that T cells also participate in

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the suppression of the response.

Many studies have been performed over the past 70 years which were designed to elicit tumor resistance following the injection of inactivated tumor cells. Haaland (5) showed that mice injected with an extract of tumor cells 30 days before challenge, demonstrated increased susceptibility to tumor growth in that the tumors grew faster and larger than those in the control mice. Kaliss (6) passively transfered this specific tumor susceptibility with the serum from treated animals and called the phenomenon "immunological enhancement".

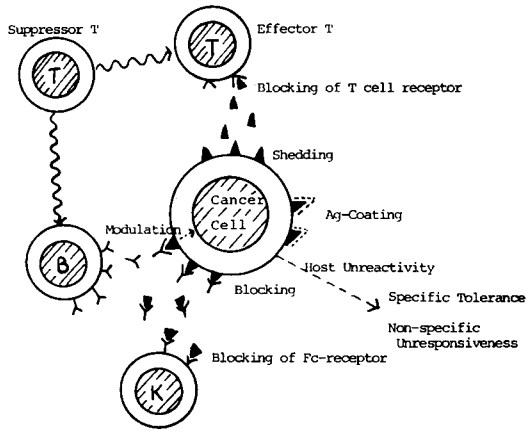

Fig. 1. The possible routes of escape for tumor cells from immunological restraint.

The mechanism of this immunological enhancement is still poorly understood. It was originally seen as a form of blocking phenomenon in which the antibody coated the tumor cells and protected them from cell-mediated immunity. Clinical observation suggested that humoral responses to tumor cells are usually associated with the earlier stages of tumor growth and not with advanced disease (7, 8). If such antibody reactions were responsible for tumor enhancement, one might have expected them to be associated with advanced disease.

Hellstroem 1969 (9) showed that serum pretreatment of the target cells can abrogate the cytolytic effects of the lymphocytes *in vitro*. This blocking activity was detected in sera, in which there is no evidence for the existence of tumor-specific antibody. On the other hand, Currie and Basham 1972 (10) showed that the lymphocytes from patients with advanced cancer need to be extensively washed before their cytolytic properties are detectable. The addition of autologous patient's serum to the washed lymphocytes abolished the newly evoked cytotoxic activity. Such inhibitory serum activity appeared to have no affinity for the tumor cell surface but appeared to act on the lymphocytes. Furthermore, when this inhibitory activity was assayed in the serum of cancer-patients, the patients with small tumor had minimal inhibitory activity but in those with more advanced disease it was readily detectable. On the nature of specific blocking and inhibition of cancerous serum, Currie and Baldwin (11, 12) suggested that antigenic moieties from the tumor cell surface which are released into the extracellular fluid and com-

plexed with specific antibody may constitute the blocking material. Moreover, evidence has accumulated, in which tumor-associated antigens such as carcinoembryonic antigen, possibly complexed with antibody, can interfere with cell-mediated response *in vivo*. In addition to this, Brown (13) showed that specifically inhibitory activity in the serum of an allogeneic mouse system appeared to be a soluble H-2 histocompatibility antigen. The concept of the existence of humoral factors capable of specifically blocking and inhibiting cell-mediated immune responses showed conclusively that at different stages of tumor progression, both immune complex and free antigen may well make vital contributions to the immunological escape of the tumor. The prime mover in such an concept is the release of specific antigenic determinants from the tumor cells in such a form that they inhibit but do not immunize. Obviously, this concept requires further scrutiny in the experiments before it can generally accepted. First, if these immunosuppressive factors in the serum of cancer-patients are circulating antigens or antigen-antibody complex, it should be clearly proved that they retain their antigenic specificity but lose their immunogenic capacity. This is not yet proved. Second, if, in advanced diseases, the circulating antigen alone is the agent responsible for abrogating immune reactions, the increase in antigen concentration should be parallel to the activity of blocking or inhibiting cell-mediated responses. Third, blocking or inhibiting of cell-mediated responses by serum of cancer-patients is not specific because of a variety of circulating antigens existing in the serum.

The evidence in favour of immunological unresponsiveness is quoted as an escape route for tumors. Two major classes of immunological deficit could be held to account for the escape of tumors. In all the human tumors so far examined, there has been no evidence proved to suggest that specific tolerance occurs because specifically cytotoxic lymphocytes are readily detectable in the blood of these patients. Furthermore, patients with a variety of tumors will react to immunization with autologous tumor cells.

CELL-MEDIATED IMMUNITY IN CANCER-PATIENTS

Clinical evidence demonstrating the importance of the patient's ability to react against these antigens is reported. The investigations of immunological capacities in cancer-patients can be grouped into two categories; those concerned with humoral antibody production and those dealing with cell-mediated reaction. Humoral antibody formation to known antigenic substances has been studied by several researchers who concluded that in most patients with carcinoma, the ability to form humoral antibodies, even in the presence of advanced disease is not greatly impaired (14, 15, 16).

Cell-mediated immunity as shown in Table 1, showed a significant im-

pairment of their ability to manifest a delayed rejection of heterologeneous skin grafts, a decreased capacity of cutaneous delayed hypersensitivity, a decreased absolute level of peripheral lymphocytes and a decreased response of the lymphocytes to non-specific mitogenic stimulation. Although immune defects have been demonstrated *in vitro* and *in vivo*, the *in vitro* response of lymphocytes to PHA has been mostly widely used for this purpose. Phytohemagglutinin exercises a powerful effect on lymphocytes in peripheral cultures, 70 to 90% undergoing transformation after a period of 72 hours. The *in vitro* reactivity of peripheral blood lymphocytes was determined in patients with carcinomas by quantitation of PHA-induced tritiated thymidine incorporation. As shown in Figure 2, our present investigation demonstrates that lymphocytes from cancer-patients have a decreased ability to respond to *in vitro* stimulation with PHA. In general, it is well known that patients with early tumors do not have any detectable immunological deficit. It is only after fairly extensive tumor growth that such defects become evident. Our result also shows that a decreased ability of lymphocytes to PHA is distinctly correlated to the stage of cancer-patients. This type of research may imply that the defects in immune reactivity are a concequence of tumor growth and do not contribute to the initial immunological escape event.

Table 1. *Cell-mediated immunity in tumor-bearing hosts*

In vivo
1. Decreased ability to reject skin homograft.
2. Decreased ability to reject tumor allograft.
3. Impairment of delayed-type hypersensitivity reactions (Tuberculin, DNCB).
4. Impairment of lymphocyte transfer reaction.
In vitro
1. Impaired responsiveness of lymphocyte to PHA.
2. Impaired Responsiveness in mixed lymphocyte culture.
3. Impairment of macrophage migration inhibition.

Incidentally, what is the essential mechanism which induces the impaired response to PHA? Three causes might be pointed out.

First, the decreased number of T-lymphocytes, second, the replacement of a population bearing an intrinsic defect and third, the existence of serum inhibitory factors.

As is shown in Table 2, the PHA responsiveness of lymphocytes from patients with advanced disease can be restored to high levels by growing the cultures in normal instead of autologous cancerous serum. These results show that a humoral factor is at least partially responsible for the impairment of cellular reactivity.

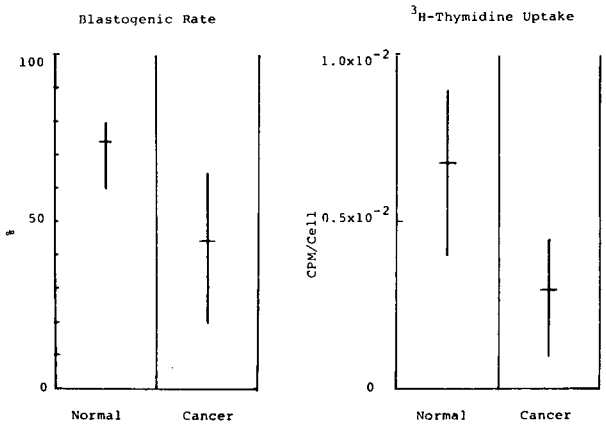

Fig. 2. Blastogenic transformation and ^{3}H-thymidine uptake of peripheral lymphocytes of cancer-patients with PHA.

Table 2. *Effects of various sera on PHA response of lymphocytes from cancer-patients*

Serum sample	Stimulation Ratio*
Autologous	0.37 ± 0.11
Homologous Cancer	0.42 ± 0.08
Homologous Normal	1.19 ± 0.25

* : Mean ± Standard deviation.

NON-SPECIFICALLY INHIBITORY ACTIVITY OF CANCEROUS SERUM ON PHA-RESPONSIVENESS OF LYMPHOCYTES *IN VITRO*

The bioassay of non-specifically inhibitory activity of cancerous serum to normal lymphocytes was carried out in glass screw-cap tubes containing one milliliter of lymphocyte suspension and 0.1 ml of serum. After an addition of PHA-P, this was incubated at 37° centrigrade in a high-humidity atomosphere of 5% carbon dioxide in air. Cancerous sera to be tested for inhibitory activity were added at a rate of 0.1 ml at the beginning of the culture. After a 70 hour incubation, 1 μCi of tritiated thymidine was added to each culture and 2 hours after the cells were collected upon millipore

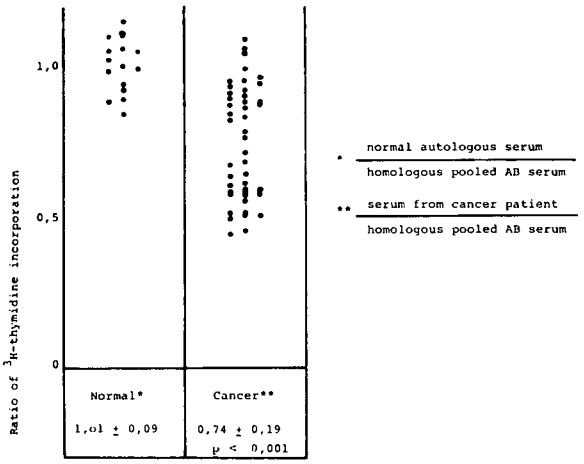

Fig. 3. Effects of sera from cancer-patients on the response of normal lymphocytes to PHA.

membranes. The radioactivity of tritiated thymidine was measured by a scintillation counter.

The inhibitory activity of the serum tested was expressed as the stimulation ratio, in comparison with the response of normal AB human serum.

Serum from patients with carcinomas added to lymphocytes from control donors appeared to be capable of inhibiting *in vitro* lymphocyte transformation, while serum of normal individuals showed no such effect. The effect of serum on normal lymphocyte reactivity was determined in patients with carcinomas by quantitation of PHA-induced tritiated thymidine incorporation.

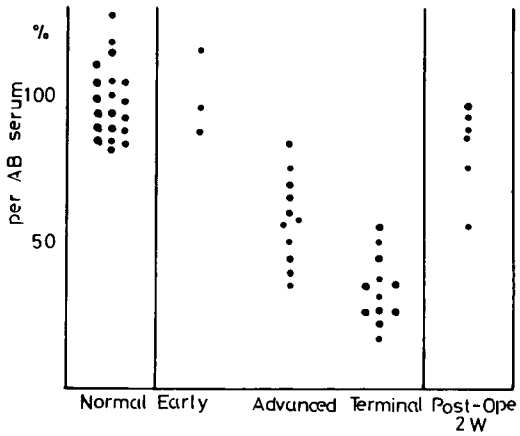

Fig. 4. Effects of sera from patients with gastric carcinoma on the response of normal lymphocytes to PHA.

Their sera suppressed normal lymphocyte reactivity as shown in Fig. 3. Sera from cancer-patients displayed a stage-related inhibitory effect shown in Fig. 4. The suppressive activity of cancerous serum was markdly diminished by the successful removal of tumor in cases of gastric carcinoma.

It is pointed out here that the immunosuppressive activity of sera from non-cancerous diseases is being recently reported in increased numbers (17,

18, 19, 20, 21). The nature and significance of the suppressive serum factors has not yet been established and the possibility exists that they might be qualitatively different in various diseases. The common trait of such factors is their ability to inhibit the responsiveness of autologous as well as homologous lymphocytes.

The development and spread of malignancy is frequently accompanied by changes in the glycoprotein composition of the serum. Outstanding features of the cancerous serum are the increase of already existing, and the appearance of new glycoproteins with alpha and beta electrophoretic mobility, characterized by a high content of carbohydrates and sialic acid. Glycoproteins are synthesized by the liver and secondarily attached to the surface of tumor cells and lymphocytes.

Although the elevations are not specific for neoplastic diseases, the serum levels of alpha-globulin are elevated in cancer and several diseased states. Therefore, the increased level of alpha- and beta-globulins may have an influence on immunosuppression, observed in PHA responses of lymphocytes.

Beginning with the report by Kamrin (22) in which he reported that alpha-globulin isolated from rat serum prolonged the survival of histoincompatible skin grafts, many investigators (23, 24, 25) have isolated humoral factors which suppress various T-cell-mediated immune responses. Riggio (26) isolated immunosuppressive alpha-globulin from pooled normal serum. This material showed the suppression of PHA-induced blastogenesis of human peripheral lymphocytes and was called as immunoregulatory alpha-globulin (IRA) by Cooperband (25).

It was recently reported that IRA is effective in non-specific suppression of immune responses dependent on T-cell function alone. Therefore, it should be considered that IRA, a naturally occuring human immunosuppressive material is increased in amount with the elevation of alpha-globulin in cancerous serum.

It was found that the serum of pregnant women frequently reduced the PHA response of washed lymphocytes obtained from normal healthy donors (27). Moreover, the inhibitory effect increased as the pregnancy progressed and reached it maximum at full term.

In pregenancies the increasing levels of circulating oestrogen and progestrone may result an imparied PHA response. Another possible inhibitory substance is alpha fetoprotein which also occurs in increasing concentrations in maternal serum as the pregnancy progresses. In addition to this, the fetal circulation contains embryo-specific protein; namely alpha fetoprotein (AFP).

The function of AFP in the fetus is unknown. One possibility is that

AFP has immunoregulatory properties which are important for the protection of the histoincompatible embryo from immunological attack by the maternal immune system.

Furthermore, the demonstration of immunosuppressive activity by AFP in relation to its occurrence in certain malignant conditions would be consistent with the association known to exist between various forms of immune deficiency and neoplastic diseases. The results of dose-response studies with purified AFP, obtained from human hepatoma tissues by the method of affinity chromatography are shown in Fig 5. The suppressive activity of purified AFP was still evident even at a concentration of 10 μg/ml. Based on our demonstration of the immunosuppressive activity of AFP, it is postulated that AFP may be one factor or possibly an important factor of many causing the non-specific immune suppression reported in many of the diseases which include ataxia telangiectasia, hepatitis, alcoholic and biliary cirrhosis and gastrointestinal carcinomas. This group of pathological conditions is of particular interest here since abnormally high AFP levels have been reported in most of these diseases.

Isoferrition which was recently recognized as one of the carcinofetoprotein is mostly elevated in the sera of malignant diseases (28). It is shown that the relationship between the immunosuppressive activity and serum ferritin concentration, measured by radioimmunoassay may closely exist in hepatic diseases including hepatomas. As shown in Fig. 6, purified ferritin from hepatoma tissues shows a definite suppression of PHA responsivenes above the concentration of 1 ng/ml.

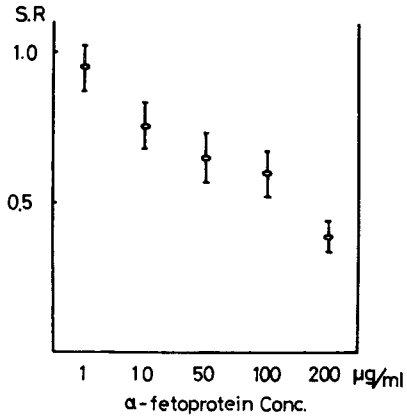

Fig. 5. Dose-responses of alpha-fetoprotein on PHA reaction of normal lymphocytes.

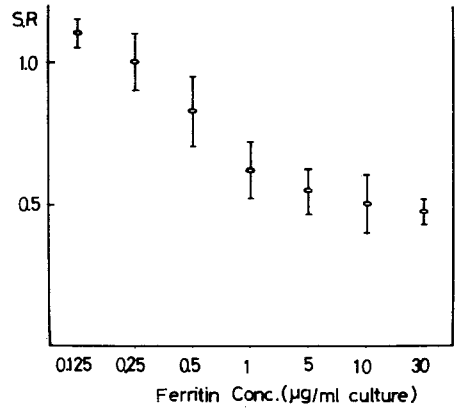

Fig. 6. Dose-response of hepatoma ferritin on PHA reaction of normal lymphocytes.

NATURE OF INHIBITORY FACTORS IN CANCEROUS SERUM

The existence of serum factors which interfere with the response of the lymphocytes to stimulation *in vitro* has been demonstrated in patients with carcinomas and also in non-cancer patients. The nature of these factors has not been established with certainty but the above mentioned evidence strongly suggests that they may differ in some of these diseases.

In order to clarify the inhibitory factors in cancer-patients, studies on the fractionation of human serum have been performed, using a microcrystalline DEAE cellulose column made by Whatman Company. The elution pat terns which separated into nine fractions of normal and cancerous sera by DEAE cellulose column chromatography are shown in Figure 7. The elution patterns of both sera were essentially similar and the repoducibility of fractionation was very good. Elution positions of the proteins have been determined using immunoelectrophoretic and agar gel diffusion analysis. Fr. 1 is composed of Ig G, Fr. 2, 3 and 4 are the beta-globulin, fraction containing transferrin, hemopexin and a small amount of Ig G. Fr. 5, 6 and 7 seem to be of the same composition, containing a large amount of albumin, a moderate amount of Ig A, alpha-2 macroglobulin a small amount of transferrin and alpha-2 HS glycoprotein. Fr. 8 and 9 are the alpha-globulin fraction, containing acid glycoprotein, haptoglobin, ceruloplasmin and Ig M. No significant difference of subfractions obtained from normal and cancerous sera is detected by immunoelectrophoretic analysis.

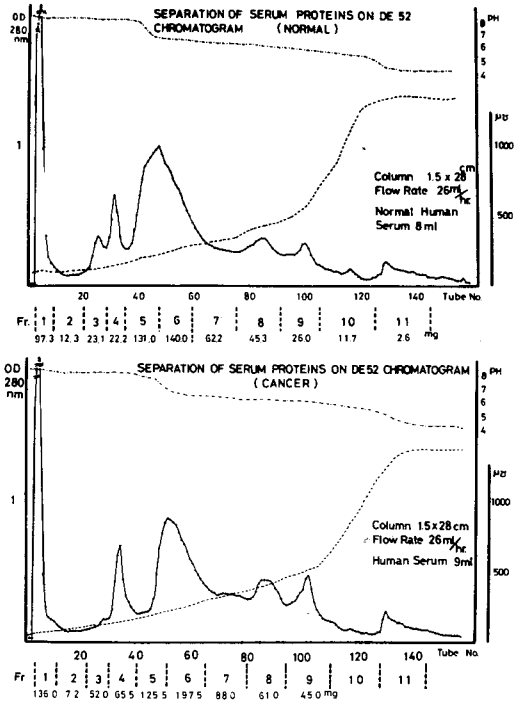

Fig. 7. Elution patterns of normal and cancerous sera by DE-52 column chromatography.

Table 3 shows the results of bioassay in nine fractions obtained from normal serum. The fractions which produced substantial suppression of PHA responses in normal lymphocytes were alpha-globulin fraction of Fr.

Table 3. *Inhibitory effects of fractionated proteins from normal sera on PHA responsiveness of normal lymphocytes*

	1.5	1.0	0.5	0.2	0.1 (mg/ml)
Fr.1	-	-	-	-	-
Fr.4	35*	-	-	-	-
Fr.5	-	-	-	-	-
Fr.6	-	-	-	-	-
Fr.7	70	55	-	-	-
Fr.8	50	38	-	-	-
Fr.9	95	70	66	-	-

* : percentage inhibition of lymphocyte responsiveness to PHA

Table 4. *Inhibitory effects of fractionated proteins from cancerous sera on PHA responsiveness of normal lymphocytes*

	1.5	1.0	0.5	0.2	0.1 (mg/ml)
Fr.1	80*	78	-	-	-
Fr.4	24	-	-	-	-
Fr.5	40	-	-	-	-
Fr.6	60	30	-	-	-
Fr.7	n.d.	94	90	-	-
Fr.8	n.d.	77	50	-	-
Fr.9	n.d.	91	68	40	-

n.d.: not determined

* : percentage inhibition of lymphocyte responsiveness to PHA

8 and 9 and beta-globulin fraction of Fr. 3 and 4. In the bioassay of the nine fractions obtained from cancerous serum, alpha- and beta-globulin fractions showed remarkable suppression of PHA responsiveness. In addition to this, the albumin fraction of cancerous sera showed a definite suppression on PHA responsiveness in normal lymphocytes as shown in Table 4. So it might be said that this suppressive activity of albumin fraction is a specific phenomenon only seen in cancer cases. Dose-dependent suppression of PHA stimulation of lymphocytes was also demonstrated in these suppressive fractions. Among them, alpha-globulin fraction showed the highest suppressive activity while the activity of albumin fraction was moderately suppressive.

DEAE cellulose column chromatography of purified AFP was preformed. It was located in the alpha-globulin fraction. Isoferritin was also located in the alpha-globulin fraction. Based on these demonstrations of the immunosuppressive activities of AFP and isoferritin, we confirmed that they are located alpha-globulin region. It is quite important to know that they are located in the alpha-globulin region because it may contain a number of inhibitory factors. It has been reported that IRA which is present in normal serum, is effective for non-specific suppression of immune responses alone dependent on the T-cell function *in vitro* and *in vivo* experiments. Since the active fractions of IRA contain primarily alpha-1 and alpha-2 globulins and since AFP and isoferritin are present in normal adult sera in very small concentrations, it cannot excluded that the suppressive effects observed with IRA preparation are due in part to low levels of AFP and isoferritin in these fractions. However, recent studies have suggested that the active moiety of IRA is a peptide which is noncovalently bound to proteins with an electrophoretic mobility in the alpha-region (29). Although our finding do not exclude the possibility of a suppressive peptide tightly bound to AFP and isoferritin, we are of the opinion that AFP and ferritin do not serve as a carrier and is probably not related to IRA.

The immunosuppressive activities of the alpha-globulin fraction and albumin fraction obtained from cancerous serum were assayed *in vivo* experiments. The plaque-forming cell response in mice injected with sheep red blood cells was remarkably inhibited by the use of both serum fractions as shown in Figure 8. This result may indicate the suppression for the helper T-cell.

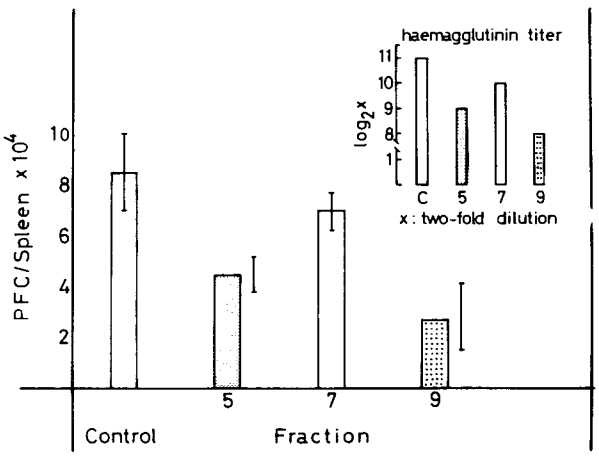

Fig. 8. Effects of fractionated serum proteins obtained from cancer-patients on PFC tests and hemagglutinin tests of mice.

What is the inhibitory mechanism of alpha-globulin and albumin fractions which were responsible for the systemic inhibition of cell-mediated immunity? Using ^{125}I labeled PHA, the effects of these two fraction to PHA binding to the surface membrane of lymphocytes were measured. Competing with PHA, the alpha-globulin fraction markedly decreased the radioactivity of PHA attached to the surface membrane of lymphocytes. On the other hand, the albumin fraction did not give any significant change to the attachment of PHA to lymphocytes as shown in Figure 9.

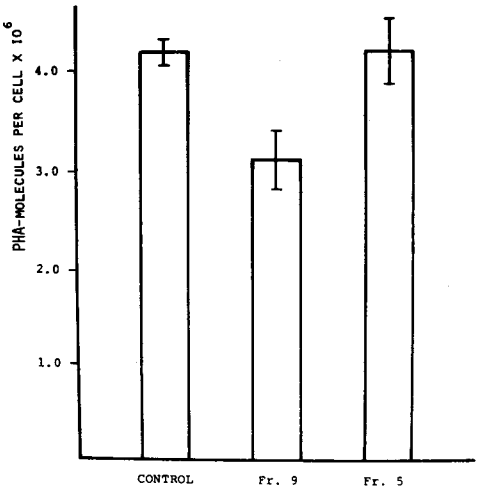

Fig. 9. Binding of ^{125}I-PHA to normal lymphocytes in the presence of fractionated serum proteins obtained from cancer-patients.

This result suggested that alpha-globulin fraction of cancerous serum becomes associated with the cell surface and exerts its inhibitory effect by interfering with the recognition of antigens by lymphocytes. The albumin fraction obtained specifically from cancerous serum may even impair the intra-cellular metabolism of lymphocytes; such as disturbance of DNA and RNA synthesis which does not comete with PHA. Therefore, the inhibitory mechanisms of both fractions may be different in their actions to lymphocytes. It is quite important that this inihibitory effect of the albumin fraction was not detected in normal serum and this fraction may well have a direct effect on the metabolism of lymphocytes.

FURTHER PURIFICATION OF CANCER-SPECIFIC FRACTION AND ITS RELATION TO TOXOHORMONE

Using Sephadex G-200 and electrofocusing fractionation, the inhibitory activity was detected in the alpha-2 macroglobulin fraction which contain cancer-specific peak with pI 3.4.

The immunosuppression associated with solid tumors is rather late in its manifestation and appears in association with other systemic signs and symptoms of malignancy. Therefore it is suggested that much of the phenomenology of tumor-bearing may be a consequence of successful tumor growth rather than being the cause of it. There appears to be some reasonable possibilities.

First malignant cells might compete with cells of the immune system for a limited supply of essential nutrients. Second, non-specific stress resulting from the presence of a large tumor might induce the host to produce immunosuppressive quantities of steroid hormone. Third, material released by the damaged host or tumor cells might induce the liver to increase production of alpha-globulin, a known immunosppressive material whose serum levels are increased in cancer-patients. Fourth, the chronic antigenic stimulation caused by constant release of tumor material into the circulation might induce the immune system to produce immunoregulatory cells or factors capable of causing non-specific immunosuppresion. Fifth, the cancer cells themselves might produce non-specific immunosuppresive material and release it into general circulation. These mechanism are not necessarily mutually exclusive and it is possible that all live may have some part in producing cancer-associated immunosuppression.

Table 5. *Soluble factors which are produced by or are extractable from tumor tissues*

1. Toxohormone	Nakahara	1949
2. Factors cytotoxic to cell in tissue culture	Watts	1963
	Sylven	1965
	Handler	1966
3. Growth-inhibitory factors	Rubin	1966
	Holmberg	1968
4. Inhibitors of DNA synthesis	Smith	1970
5. Inhibitors of blastogenesis	Anderson	1972

There have been numerous reports on soluble factors which are produced by tumor and which have a profound effect upon the morphology or physiology of other tissues (30, 31, 32, 33). These include toxohormone, factors cytotoxic to cells in tissue culture, growth inhibitory factors, inhibitors of DNA synthesis and inihibitors of blastogenesis as shown in Table 5. Many these factors have a definite effect on the cells of the immune system.

It is known that cell-free fluid from cancer-patients can suppress the PFC response against sheep red cells *in vivo* (34, 35). The tumor-derived immunosuppressants should not be considered to be specific for the immune system. They may well be responsible also for anemia and general wasting often seen in patients with advanced cancer. The suggestion that localized cancer may biologically secrete an active material which is responsible for various systemic abnormalities often found in association with cancer was first made by Greenstein (36).

Since that time, the excellent pioneering work by Nakahara (37, 38) demonstrated that material capable of causing a variety of biological effects when injected into the animals, can be extracted from many different types

of tumors.

Crude toxohormone, ethanol precipitate of tumor tissues of gastric carcinoma, obtained by the method of Nakagawa (39) was fractionated by column chromatography using DEAE-Sephadex A-50 as the bed material. A typical chromatogram is shown in Figure 10. The fraction 2 which was a purified toxohormone, showed a definite decrease of catalase activity of mice liver when injected intraperitoneally. The purified toxohormone was markedly suppressive to the PHA responsiveness of normal lymphocytes in a dose of 50 ug/ml *in vitro* as shown in Figure 11. On the other hand, the soluble

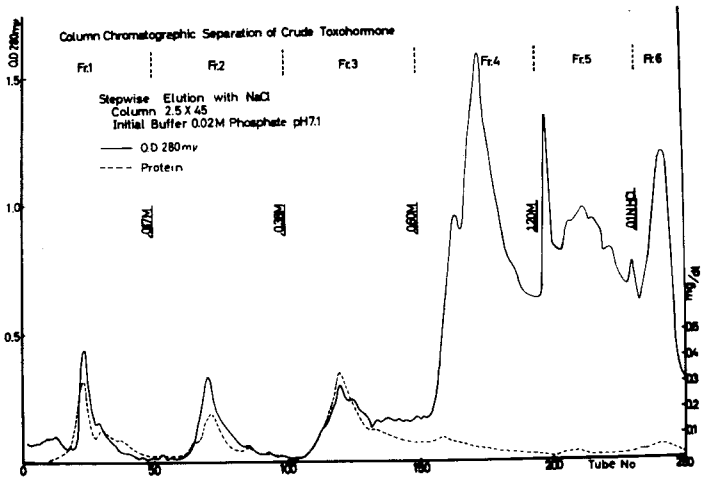

Fig. 10. Column chromatographic separation of crude toxohormone.

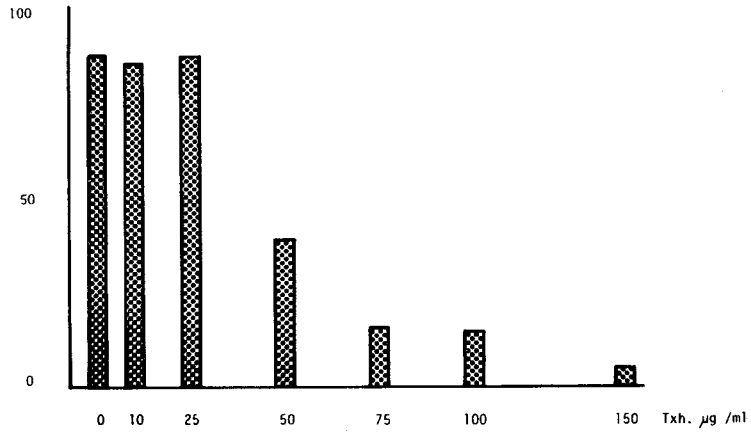

Fig. 11. Effects of purified toxohormone on the PHA responsiveness of normal lymphocytes.

material extracted from normal stomach tissues by the same method, showed no remarkable effect *in vitro*. Using ^{125}I labeled PHA, the effect of purified toxohormone on PHA binding of surface membrane of lymphocytes was measured. The administration of toxohormone had no significant effect upon the PHA binding to the lymphocytes.

This result is quite similar to the effect of albumin fraction obtained from cancerous serum.

In order to reproduce experimentally the systemic effects characteristic to the tumor-bearers, two mg of purified toxohormone were each dissolved in 5 ml of distilled water and injected subcutaneously over a period of 5 days, five times a day, into individual rats. The lymphocytes obtained from toxohormone treated rats showed a remarkable decrease of PHA responsiveness. The systemic effects of purified toxohormone on the iron metabolism of normal rats were already reported (40, 41, 42) to shown a remarkable decrease of serum iron concentration, a profound decrease of hepatic ferritin, a marked lowering of intestinal iron adsorption and disturbance of utilization of colloid iron in the reticuloendothelial system.

CONCLUSION

In summary, We would like to emphasize that the cellular immunity is probably the most important aspect of tumor immunity. In spite of the existence of tumor specific antigen in human cancer, a tumor grows. For this apparent paradox, two explanations are prepared. One is a specific regulation by antigen and antibody. The other is a non-specifically immunodeficit state in the host. Regarding specific humoral regulation, there are some problem to be solved to confirm the specificity. Another problem is the main cause non-specific unresponsiveness in the host immune reaction. The nhibitory effect of albumin fraction, especially alpha-2 macroglobulin subfraction was not detected in normal serum and it closely resembled that of toxohormone which is extracted from cancer tissues. However tumor-derived immunosuppressants should not be considered to be specific for the immune system; they however may well be responsible for anemia and general wasting often seen in patients with advanced cancer.

It might be concluded that the albumin fraction, especially alpha-2 macroglobulin subfraction in cancerous serum probably contains inihibitory substances released from cancer tissues.

Further purification and identification of these inhibitory factors must be performed in cancer-patients.

The all-over results indicate that cell-mediated immunity may be regulated by a variety of humoral factors. In cancer-patients it may be possibly

altered by specific and non-specific factors not present normal serum.

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