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Local Immunotherapy of Mouse Mammary Carcinoma by Hetorologous Antibodies Reactive Aginst Serum Alpha-Globulin Components

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SUMMARY

Antibodies raised against the whole alpha-globulin fraction of mice serum killed mouse mammary carcinoma tumor cells and normal spleen cells by icubation and abrogated the growth of transplanted mammary carcinoma by local injection at the tumor site. Immunoelectrophoreticaly, 3-5 different components could be detected in the isolated serum fraction and all of them cross reacted in the immunodiffusion analyses with components included in serum-free tumor or normal crude mouse tissue extracts. Local treatment of the tumor by heterologous antibodies raised against the serum alpha-globulin or the tissue alpha-globulin equivalent was effective at the early stages of the disease development without systemic adverse reactions.

Key words: Alphaglobulins, Antibodies, Cancer therapy

INTRODUCTION

It has been previously demonstrated that major fraction of the serum proteins, the immunoglobulins, are acting as anti-immunoglobulins which interfere with the cytotoxicity of the former against neoplastic cells (8, 9). It has also been claimed that tumor components accumulated in the blood may similarly block anti-tumor cytotoxic antibodies (1, 8). Furthermore, in sheep bronchoalveolar carcinoma alpha-globulin equivalents of tumorous and normal tissues existed in increased amount in the tumor tissue and the peripheral blood of the tumor affected animals (7, 10). In the present study we have prepared heterologous hyperimmune sera against electrophoreticaly isolated whole alpha-globulin region of mice serum and against serum-free tumor and normal mice tissue. Cross-immunity were found between the serum isolate and the serum-free tissue extracts. Various antibody preparations raised against these complementary alpha-globulin elements, when

injected at the site of mouse mammary carcinoma inoculum in BALB/c mice, abrogated the growth of the tumor cells.

MATERIALS AND METHODS

Mouse mammary carcinoma

Mouse mammary carcinoma was developed spontaneously in our BALB/c mice colony (6) and has been repeatedly transplanted and preserved in this strain by the following procedure : The tumor mass growing subcutaneously was minced and stirred for 10 minutes in Trypsin-EDTA solution (NaCl 8 g+KCl 0.4 g+glucose 1 g+$NaHCO_2$ 2 g+trypsin 2.5 g+EDTA 0.5 g+Phenol red−1% 2 m*l*+H_2O to 1 L, adjusted to pH 7.7 by 1N NaOH). The suspension was centrifuged lightly and the sediment resuspended in Dulbeco's MEM medium supplemented with fetal calf serum (10%) and antibiotics. The cell counts in the final suspension was determined in haemacytometer using Trypan Blue as the staining solution. The suspension, 0.2 m*l* containing 2×10^5 live tumor cells was injected subcutaneously into the gluteal region. The tumor mass could be palpated 9-10 days post inoculation and the animals died on day 16 post inoculation due to extensive spread of the disease.

Separation of serum protein components

Non-denaturative 7.5% polyacrylamide gel electrophoresis, immunoelectrophoresis, immunodiffusion and paper electrophoresis were used by the standard methods previously described (2, 3, 5, 6) in analytical stadies of serum proteins. The serum was run in the polyacrylamide gel which was then cut out according to the major visible protein components corresponding with a reference gel containing stained mouse serum protein pattern. The serum components were extracted from the gel and pooled samples were analysed by the above mentioned methods and thier relation to the classical major serum proteins was determined. Pooled preparations, each of which possessing the characteristic features of one of the major serum proteins (albumin, alpha-, beta-, gammaglobulin) were used separately as the antigens in the heteroimmunization experiments performed by the methods described (3, 5, 6).

Antiserum preparation

Antisera for each of the serum protein fractions were raised in three rabbits and three chickens. Each of the animals provided serum samples which were pooled to form a single lot of antiserum so that for each of the serum protein fractions six lots of antisera were prepared and used in the various experimental sets. The antisera were adjusted either by concentration (lyophilization) or by

dilution (in 1% PBS) to the concentration of 2 mg of gammaglobulins per m*l* of serum. Isolated gammaglobulin obtained from each of the antisera lots by the salting out procedure was dissolved in saline solution to the concentration of 2 mg per m*l*. The resulted solutions were used to in the various experimental sets.

Serum-free tissue extracts and thier antisera

Serum-free tumor and normal tissue extracts were obtained by the acid glycine elution technique previously described (5, 6, 7). Antisera against the extracts were prepared as before (7) and were used in the experimental sets after being processed as mentioned above.

Incubation experiments

Incubation of antibodies and the control solutions with the tumor and the normal cell suspensions was done at 37℃ for 2 hours. Three m*l* of each of the cell suspensions containing 1×10^6/m*l* live cells was supplemented by various concentrations of the above mentioned antibodies and control preparations. Cell counts were done with Trypan Blue as above. Normal nucleated spleen cells were obtained by intrasplenic injections of saline solution.

Inoculation experiments

In each of the experimental sets, groups of six adult femal BALB/c mice in duplicates were inoculated with the tumor cell suspension and each group was treated by one of the treatment or the control preparations. Dilution of the preparations was performed with Dulbeco's medium. Injections at the inoculation site were done 3, 6, and 9 days post inoculation.

RESULTS

Of all the hyperimmune sera and the hyperimmune immunoglobulin isolates, only two, the one raised against the serum whole alpha-globulin fraction and the one raised against the serum-free tissue extracts were cytotoxic to the mammary carcinoma cells either by incubation or inoculation. They were also toxic to the normal spleen nucleated cells. These results are summarized in Table 1. There were no significent differences among the effects of the comparable preparations so that a characteristic experimental set using rabbit serum and antiserum is presented. Several remarks should be noted. At the concentration of 2.5% of antibodies spleen cells were not affected in comparison with the incubated tumor cells. In animals treated by the antibody preparation the tumor masses, when developed, could be palpated at days 10-14 and the animals died on days 25-35. The similarities in the effect of the antisera raised against the serum alpha-globulin and the serum-free

Table 1. *The effect of anti serum protein fractions antibodies on mouse mammary carcinoma tumour cells and normal spleen cells by incubation or during inoculated tumour growth*

Experimental system	Treatment	Results
Tumour cell suspension	10% antialpha-globulin	100% cell death
〃	5% 〃	100% 〃
〃	2.5% 〃	75% 〃
〃	10% intact serum	0% 〃
〃	Dulbecco's medium	0% 〃
〃	10% antigamma-globulin	0% 〃
〃	10% antialbumin	0% 〃
Spleen cell suspension	10% antialpha-globulin	100% cell death
〃	5% 〃	100% 〃
〃	2.5% 〃	25% 〃
〃	10% intact serum	20% 〃
〃	10% Dulbecco's medium	20% 〃
Tumour growth *in vivo*	10% antialpha-globulin	2/12 animal death
〃	5% 〃	6/12 〃
〃	intact serum	12/12 〃
〃	Dulbecco's medium	12/12 〃
〃	10% antigamma-globulin	12/12 〃
〃	10% antialbumin	12/12 〃

tissue extracts could be explained by the immunodiffusion and the immunoelectrophoretic analyses. Both antibody preparations precipitated from the serum the same 3–5 alpha-globulin fractions and non of the others.

DISCUSSION

This study concluded our long term experiments concerning the effect of antibodies raised against different serum proteins on tumor growth in mice. We have previously demonstrated that hyperimmune serum against mouse immunoglobulin-G accelerated the growth and development of Ehrlich ascites tumor (Landscutz) in the ascitic or the solid forms (4). In the present study we have detected antigenic identity between the serum alpha-globulins and cells from normal and tumorous mice tissues. Thus, specific antibodies raised against the serum alpha-globulins affected also the growth of the tumor cells. These antibodies are

cytotoxic to the normal cells as well, however, by local administration at the site of the tumor inoculation no systemic adverse reaction occurred. It seems likely that the same mechanism may acts also in man. Nevertheless, the presence in the peripheral blood of serum proteins antigenically related to the tumor tissue might interfere with the effect of natural antibodies in cancer patient.

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