Detection of species – and tissue - unrestricted conformation – dependent tumor associated antigen(s) in immune complexes from plasma of tumor affected cattle and buffaloes

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Citation

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Abstract

Rabbit hyperimmune serum against intact whole immune complexes from a mammary tumor affected dog gave positive reactivity in dot ELISA with plasma from cattle and buffaloes affected with various tumors compared to sera against the antigen – rich and the antibody – rich fractions of dissociated circulating immune complexes (CICs). These results suggest a possibility of existence of species – and tissue - unrestricted tumor associated antigens (TAAs) as conformation - dependent epitope(s) in CICs in bovine tumors.

INTRODUCTION

Circulating immune complexes in tumor affected animals may be a rich source of tumor antigens capable of eliciting antibody response. The present study was aimed at exploring this possibility with blood plasma from tumor affected cattle and buffaloes.

MATERIALS AND METHODS

The present study was conducted on samples of blood plasma from 3 cattle and 6 buffaloes with histopathologically confirmed tumors of different kinds and from normal healthy animals.

Precipitation of circulating immune complexes: CICs were precipitated from plasma of a mammary tumor affected dog by incubating the plasma with equal volume of 6% Polyethylene Glycol 6000 (PEG 6000) for 1 hour at 40C and centrifugation at 1000 g for 20 minutes. The supernatant was removed and the pellet was washed twice with 3% PEG 6000 in PBS (pH 7.4). The precipitated CICs were resuspended in 1.5 ml of PBS.

Dissociation of immune complexes: The CICs were dissociated by 8M Urea. Fractionation of immune complexes: Dissociated CICs were fractionated by ion – exchange chromatography using DEAE cellulose resin. The fractions obtained before and after elution were pooled separately and lyophilized. The two fractions, designated as fraction I and fraction II, were resuspended in 0.5 ml PBS each.

Hyperimmune sera: Healthy albino rabbits were used for raising hyperimmune sera against the whole immune complexes and fractionated CICs from plasma of a dog with histopathologically confirmed mammary tumor. Suspension (0.5 ml) of CICs or fractions of CICs was emulsified with an equal volume of Freund's Complete Adjuvant and injected intradermally in rabbits. First booster injection along with Freund's Incomplete Adjuvant (FIA) was given after two weeks and the second booster along with FIA was given after another week. Serum was collected a week after the second booster injection. Hyperimmune serum against the immune complexes of tumor bearing dog was adsorbed for half an hour with immune complexes obtained from plasma of a normal healthy dog.

Immunological analyses: All the plasma samples of tumor affected cattle and buffaloes were analyzed by ELISA using hyperimmune sera against whole immune complexes and fractionated CICs of a mammary tumor bearing dog to look for the presence of any possible tumor associated antigens. Indirect dot ELISA was performed. Plasma samples (1 l each) from tumor bearing bovines were coated on nitrocellulose membrane dipsticks and the dipsticks were

dried for 1 hour at room temperature. The first antibody used was the rabbit hyperimmune serum (diluted 1:30) and the second antibody was goat anti-rabbit IgG –HRPO conjugated (diluted 1: 60). The substrate mixture contained 5 mg DAB/10 ml Phosphate buffer and 10 l H_2O_2. The development of brown colored dot indicated a positive reaction. Along with the samples from tumor affected animals, plasma samples from normal healthy animals were also used as controls.

RESULTS

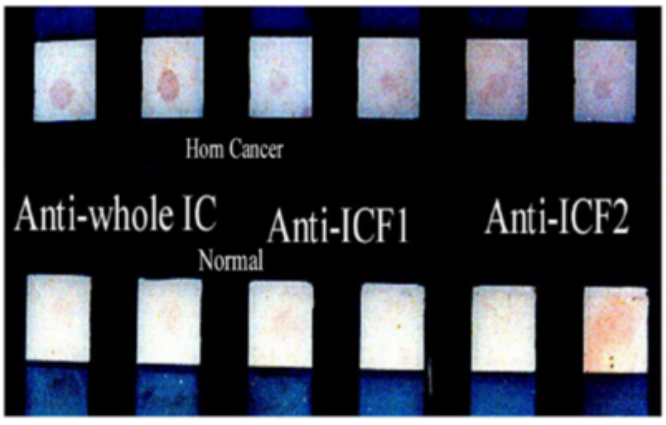
Plasma samples: Plasma samples were collected from 3 cattle and 6 buffaloes with various tumors. Cattle tumors included one case each of horn cancer, gum tumor and tumor of nasal cavity, respectively. Buffalo tumors included one case each of udder, teat and cervix tumors and three cases of eye tumors.

Dot ELISA of Rabbit anti-canine whole immune complex (anti-whole IC) serum against plasma of tumor bearing bovines:

Cattle tumors: The rabbit anti- whole IC serum gave a ++ reactivity with plasma samples of tumor bearing cattle and a + reactivity with plasma of normal healthy controls (Fig. 1; Table 1.).

Figure 1

Figure 1: Dot ELISA reactivity of hyperimmune sera against canine immune complexes and their fractions with plasma from tumor bearing bovines. Note the high reactivity of anti-whole IC serum compared to sera against IC fractions.



Buffalo tumors: The rabbit anti- whole IC serum gave a +++ reactivity with 33.3% of the plasma samples and a ++ reactivity with 66.6% of the plasma samples of tumor bearing buffaloes and a + reactivity with plasma of normal healthy controls (Table 2.).

Dot ELISA of Rabbit anti-canine immune complex fraction I (anti- ICF_1) serum against plasma of tumor bearing bovines:

Cattle tumors: The rabbit anti- ICF_1 serum gave the same (+) reactivity in all the samples of tumor bearing cattle as that of normal healthy controls (Fig. 1; Table 1.).

Figure 2

Table 1: Dot ELISA reactivity of plasma from cattle affected with various tumors to rabbit anti-canine immune complex (IC) sera.

S. no.	Tumor type	Anti-whole IC	Anti-ICF_1	Anti-ICF_2
1	Horn cancer	++	+	+
2	Gum tumor	++	+	+
3	Tumor in nasal cavity	++	+	+

Reactivity of plasma from normal controls = +

Buffalo tumors: The rabbit anti- ICF_1 serum gave a ++ reactivity with 33.3% of the plasma samples of tumor bearing buffaloes while 66.6% of the plasma samples of tumor bearing buffaloes gave the same (+) reactivity as that of plasma of normal healthy controls (Table 2.).

Figure 3

Table 2: Dot ELISA reactivity of plasma from buffaloes affected with various tumors to rabbit anti-canine immune complex (IC) sera.

S. no.	Tumor type	Anti-whole IC	Anti-ICF_1	Anti-ICF_2
1	Udder tumor	+++	++	+
2	Eye tumor	+++	++	+
3	Eye tumor	++	+	+
4	Eye tumor	++	+	+
5	Teat tumor	++	+	+
6	Cervical tumor	++	+	+

Reactivity of plasma from normal controls = +

Dot ELISA of Rabbit anti-canine immune complex fraction II (anti- ICF_2) serum against plasma of tumor bearing bovines: The rabbit anti- ICF_2 serum gave the same (+) reactivity with plasma from all the tumor bearing cattle and buffaloes as that of normal healthy controls (Tables 1 and 2).

DISCUSSION

In the present study, the rabbit hyperimmune serum against whole immune complexes from plasma of a dog with mammary tumor when tested against plasma of tumor affected cattle and buffaloes was found to give a low reactivity with plasma from tumor bearing cattle. In contrast, the reactivity was high with one third of the samples and low

with two thirds of the samples of plasma from tumor bearing buffaloes. This difference in reactivity in only a proportion of samples within the same species may possibly be due to the different clinical stages and immunological status of tumor in different animals. However, the hyperimmune serum against the antigen – rich fraction I of the dissociated CICs gave a background reactivity comparable to the healthy controls in majority of the samples from animals with tumors in both, cattle and buffaloes.

Cronin et al. (1982) purified CICs from pleural effusions of patients with squamous and adenocarcinomas of lung. The hyperimmune sera raised against the antigen – rich portion of CICs were found to stain the tumors. The presence of TAAs in CICs has also been reported in melanoma patients by Gupta and Morton (1983). The antigenic portion of the dissociated complex was shown to react with allogeneic sera and with a rabbit anti-melanoma serum. Thus, positive reactivity of the plasma samples from tumor affected animals with serum against F_1 fraction of CICs compared to the normal controls should indicate the presence of circulatory free antigen in the patients. However, the low positive reactivity with hyperimmune serum against F_1 fraction of CICs observed in our study, may indicate an absence or a low level of circulating free antigen. The higher positive reactivity in case of anti-whole IC serum compared to anti-ICF_1 or anti-ICF_2 sera may possibly indicate the presence of conformational epitope(s) formed due to the antigen – antibody interaction which may be found in intact CICs but absent in circulating free antigen.

Chester et al. (1994) used monoclonal antibodies for the detection of free and immune complexed antigen in the sera of patients with colon carcinoma. They concluded that the analysis of both, IC bound and free circulating antigen, is a more sensitive indicator of the disease condition. In our study, the samples from tumor bearing bovines gave a negligible or background reactivity against the hyperimmune serum to ICF_2 (antibody – rich) fraction of CICs comparable to that of healthy controls. The inability to detect tumor specific antibodies in plasma with these sera may possibly be due to the lack of humoral immune response against tumor antigens in these animals.

The present studies indicate that antibodies against the whole IC could detect the tumor associated antigen(s) in plasma of tumor affected bovines. The failure of antibodies to the antigen – rich and antibody – rich fractions of dissociated immune complexes to differentiate between plasma from tumor bearing and normal animals could possibly imply that the putative tumor associated antigen(s) may not exist as independent linear epitope(s). Instead, they may possibly exist as conformational epitope(s) formed by the binding of antigen and antibody. Such putative tumor marker(s) seem to be unrestricted to the tissue type and species concerned since the hyperimmune sera against CICs of dog with mammary tumor reacted with the plasma of cattle and buffaloes with various tumors.

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