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

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 40°C 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 101 H₂O₂. 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.).

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 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.).

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

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 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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References
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