ISSN : 0974 - 7532 Volume 6 Issue 6,7

Trade Science Inc.

Research & Reviews in BioSciences

Regular Paper

RRBS, 6(6,7), 2012 [187-190]

Detection of conformation-dependent tumor associated antigens in immune complexes from plasma of mammary and venereal tumor affected dogs

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Received: 6th May, 2012 ; Accepted: 6th June, 2012

ABSTRACT

Hyperimmune serum against intact whole immune complexes from mammary tumor affected dog gave strong positive reactivity in dot ELISA with plasma from dogs affected with mammary, venereal and other tumors compared to sera against the antigen - rich and the antibody - rich fractions of dissociated circulating immune complexes. These results suggest a possibility of existence of tumor associated antigens (TAAs) as conformation - dependent epitope (s) in circulating immune complexes in canine tumors.

KEYWORDS

Conformation-dependent epitopes;
Tumor associated antigens;
Canine tumors.

INTRODUCTION

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

MATERIALS AND METHODS

The present studies were conducted on samples of blood plasma from dogs with histopathologically confirmed tumors and normal healthy dogs.

Precipitation of circulating immune complexes (CICs)

CICs were precipitated from plasma by incubating the plasma with equal volume of 6% Polyethylene glycol (PEG-6000), incubating for 1 hour at 4^0C and centrifugation at 1000 g for 20 minutes. The supernatant was removed and the pellet was washed twice with 3% PEG in PBS (pH 7.4). These precipitated CICs were resuspended in 1.5 ml of PBS.

Dissociation of circulating immune complexes

The suspension of CICs was mixed with 8M Urea to dissociate the CICs.

Fractionation of circulating immune complexes

Dissociated CICs were fractionated by ion –

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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 (pH 7.4) 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 mammary tumor. CIC suspension (0.5 ml) was emulsified with an equal volume of Freund's Complete Adjuvant (FCA) 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 tumor immune complexes was adsorbed for half an hour with non – tumor immune complexes obtained from 9 ml plasma from a normal healthy dog.

Immunological analyses

All the plasma samples were analyzed by ELISA using hyperimmune sera against canine mammary tumor whole immune complexes and fractionated CICs to look for the presence of any possible tumor associated antigens. An indirect dot ELISA was standardized. Plasma samples (1 ml each) were coated on the 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 5mg DAB/10ml PBM and 10 ml H_2O_2. The development of brown colored dot indicated a positive reaction. Along with the samples, normal plasma from a healthy dog was also used as a negative control.

RESULTS AND DISCUSSION

Samples from tumor bearing animals

In the present study, samples from a total of 59 cases of various tumors from dogs were collected. These included 34 dogs with mammary tumors, 20 with transmissible venereal tumors and 5 with tumors of connective tissue on the chest and cheek, respectively. Most of the cases of mammary tumor showed the histopathological picture of adenocarcinoma which, in some cases, was mixed with cystic structures or with fibroma or myxoma. In two cases, along with the adenocarcinoma, chondroma was also found. One mammary tumor showed reticular cell sarcoma. The venereal tumors were mostly lymphosarcomas which, in a few cases, were mixed with fibromas. Four cases of leiomyoma were also found in this group. Included in the miscellaneous tumors were 3 connective tissue tumors, one hemangioma and one fibroma, respectively.

Dot ELISA of rabbit anti-whole immune complex (anti-whole IC) serum against plasma of tumor bearing dogs.

The Dot ELISA of anti-whole IC serum versus plasma revealed a high positive reactivity (+ + +) in 67.64% samples in mammary tumor (Figure 1, TABLE 1), 70% samples in venereal granuloma (Figure 2, TABLE 2), and 40% samples in miscellaneous tumor bearing dogs (TABLE 3), respectively.

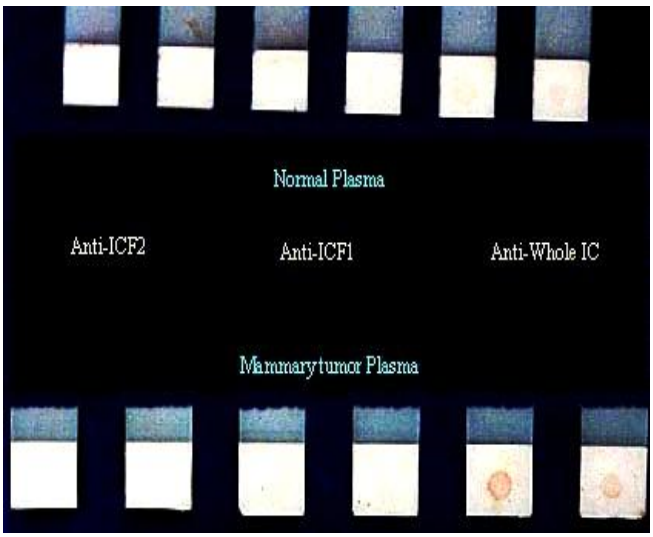

Figure 1 : Dot ELISA of anti - immune complex serum against plasma of mammary tumor bearing dogs.

A low positive reactivity (+ +) was observed in 33.36% samples from mammary tumor, 30% samples from venereal granuloma and 60% samples from miscellaneous tumor bearing dogs, respectively compared to the background reactivity (+) of plasma from normal healthy dogs.

Overall reactivity

The rabbit anti-whole IC serum tested

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TABLE 1 : Reactivity of plasma from mammary tumor affected dogs with anti-immune complex sera in Dot ELISA.

S. no.	Anti-whole IC	Anti-ICF_1	S.no.	Anti-whole IC	Anti-CF_1
1	++	++	18	+++	++
2	++	+	19	+++	+++
3	+++	++	20	+++	++
4	+++	++	21	+++	++
5	++	+	22	++	++
6	+++	+++	23	+++	++
7	++	++	24	+++	++
8	+++	++	25	+++	++
9	+++	+++	26	+++	++
10	+++	++	27	+++	+++
11	++	++	28	+++	++
12	++	++	29	++	++
13	+++	++	30	++	++
14	+++	++	31	++	+
15	+++	++	32	+++	++
16	++	++	33	++	++
17	+++	++	34	+++	++

Reactivity of normal control plasma = +

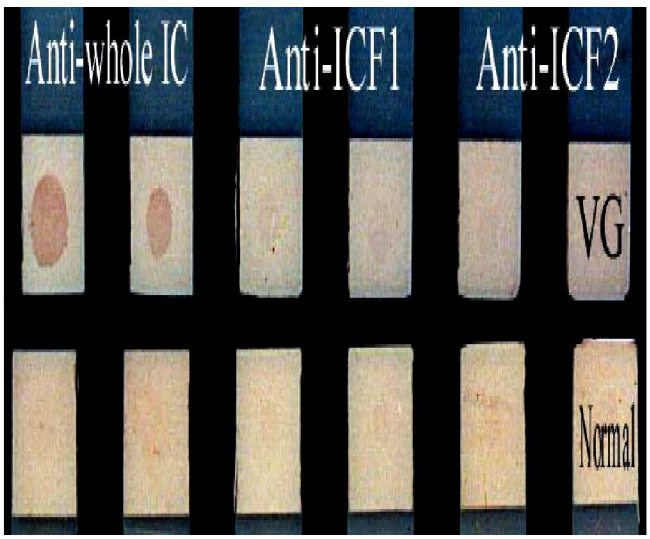

Figure 2 : Dot ELISA of anti - immune complex serum against plasma of venereal granuloma bearing dogs

against 59 plasma samples from dogs with various tumors showed a high positive (+ + +) reactivity in 66.10% of samples from tumor bearing animals and a low positive (+ +) reactivity in 33.90% samples compared to the background (+) reactivity of normal healthy controls.

Dot ELISA of rabbit anti- canine immune complex fraction I (anti-ICF_1) serum against plasma of tumor bearing dogs

The Dot ELISA of anti-ICF_1 serum versus

TABLE 2 : Reactivity of plasma from venereal granuloma affected dogs with anti-immune complex sera in Dot ELISA.

S. no.	Anti-whole IC	Anti-ICF_1	S. no.	Anti-whole IC	Anti-ICF_1
1	+++	++	11	+++	++
2	++	+	12	+++	++
3	+++	++	13	+++	++
4	+++	++	14	+++	++
5	+++	+	15	++	+
6	+++	++	16	++	+
7	+++	++	17	++	+
8	+++	+	18	++	+
9	+++	++	19	+++	++
10	++	++	20	+++	++

Reactivity of normal control plasma = +

TABLE 3 : Reactivity of plasma from dogs affected with various tumors to anti-immune complex sera in dot ELISA

S. no.	Tumor type	Anti-whole IC	Anti-ICF_1
1	Tumor of connective tissue (leg)	+ + +	+ +
2	Tumor of connective tissue (leg)	+ + +	+ +
3	Tumor of connective tissue (leg)	+ +	+ +
4	Tumor on chest	+ +	+ +
5	Tumor on cheek	+ +	+ +

Reactivity of normal control plasma = +

plasma revealed a high positive reactivity (+ + +) in only 8.82% samples in mammary tumor (Figure..1, TABLE 1) affected dogs. In contrast, a low positive reactivity (+ +) was observed in 79.41% samples from mammary tumor (Figure 1, TABLE 1), 65% samples from venereal granuloma (Figure 2, TABLE 2), and 40% samples from miscellaneous tumor bearing dogs, respectively. A background reactivity (+) as in negative controls was found in 11.76% samples from mammary tumor, 35% samples from venereal granuloma, and 60% samples from miscellaneous tumor affected dogs, respectively.

Overall reactivity

The rabbit anti-ICF_1 serum tested against 59 plasma samples from dogs with various tumors showed that 5.08% of samples from tumor bearing animals had a high positive (+ + +) reactivity, 71.19% samples had a low positive (+ +) reactivity and 30.72% samples had the same reactivity as background (+) reactivity of normal healthy controls.

Dot ELISA of rabbit anti- canine immune complex fraction II (anti-ICF_2) serum against plasma of tumor bearing dogs:

The Dot ELISA of anti-ICF_2 serum versus

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plasma revealed a high positive reactivity (+ + +) in only 5.88% samples in mammary tumor affected dogs. In contrast, a low positive reactivity (+ +) was observed in 17.64% samples from mammary tumor bearing dogs only. A background reactivity (+) as in negative controls was found in 78.47% samples from mammary tumor, and 100% samples from venereal granuloma, and miscellaneous tumor affected dogs, respectively.

Overall reactivity

The rabbit anti-ICF_2 serum tested against 59 plasma samples from dogs with various tumors showed that 3.39% of samples from tumor bearing animals had a high positive (+ + +) reactivity, 10.18% samples had a low positive (+ +) reactivity and 86.44% samples had the same reactivity as background (+) reactivity of normal healthy controls.

In the present study, the hyperimmune serum raised against whole immune complex from plasma of a dog with mammary tumor, when tested against plasma samples, was found to react very strongly with plasma from tumor bearing dogs in majority of cases of mammary tumor and venereal granuloma whereas, the reactivity was moderate in majority of samples of plasma from dogs with miscellaneous tumors and only a lesser proportion of the samples showed a high positivity. Overall, the reactivity was high in most of the samples from dogs with various tumors. However, the hyperimmune serum against the antigen – rich fraction I of the dissociated CICs gave a low positive reactivity in majority of the cases of mammary tumor, venereal granuloma and miscellaneous tumors of dogs.

In an earlier study, the hyperimmune sera raised against the antigen – rich portion of CICs purified from pleural effusions of patients with squamous and adenocarcinomas of lung were found to stain the tumors[1]. The presence of TAAs in CICs has also been reported in melanoma patients[2]. The antigenic portion of the dissociated complex was shown to react with allogeneic sera and with a rabbit anti-melanoma serum. Positive reactivity of the plasma samples from tumor affected animals with serum against F1 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 F1 fraction of CICs observed in our study, may indicate a low level of the circulating free antigen. The higher positive reactivity in case of anti-whole IC serum compared to anti-ICF sera may indicate the presence of some conformational epitope (s) formed due to the antigen – antibody interaction which may be found in intact CICs but absent in circulatory free antigen.

Chester et al. used monoclonal antibodies for the detection of free and immune complexed antigen in the sera of patients with colon carcinoma and claimed that the analysis of both, IC bound and free circulating antigen, is a more sensitive indicator of the disease condition[3].

In our present study, most of the samples from mammary tumor, venereal granuloma and miscellaneous tumors of dogs gave a negligible reactivity against the hyperimmune serum to ICF_2 (antibody – rich) fraction of CICs as compared to the healthy controls. The inability to detect tumor specific antibodies may be due to the lack of humoral immune response against tumor antigens.

The present studies indicate that antibodies against the whole IC could pick the tumor associated antigen (s) from plasma in dogs with mammary tumor and venereal granuloma. 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, it may possibly exist as conformational epitope (s) formed by the binding of antigen and antibody. Such putative tumor marker (s) seem to be restricted to the tissue type and species concerned.

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