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Immunosuppressive Proteins in the Serum from the Dog with Squamous Cell Carcinoma

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(Received 5 April 1984/Accepted 15 September 1984)

ABSTRACT. The lymphocyte blastogenesis to phytohemagglutinin was inhibited by addition of the serum from the dog with squamous cell carcinoma. The immunosuppressive factor in the serum was investigated by the use of isoelectrofocusing (IEF) and two-dimensional gel electrophoresis (TDGE). Abnormal proteins were detected in the tumor-bearing serum. The abnormal proteins migrated in acidic region, and had an isoelectric point of around 3.2. These acidic proteins were separated into two protein bands by TDGE analysis, and closely resembled the immunosuppressive acid protein (IAP) which was found in the sera from human patients and mice with tumors.—**KEY WORDS:** immunosuppressive factors, tumor-bearing dog.

Jpn. J. Vet. Sci. 47(1): 121–123, 1985

Immunosuppression is often accompanied by malignancy in both human and animals with both hyporesponse lymphocytes and abnormalities of the serum being reported [6]. A number of groups have reported that the serum from some cancer patients had been suppressive of lymphocyte blastogenesis *in vitro* [1, 4]. This immunodepression has been attributed to the presence of suppressive factors such as immunoregulatory alpha-globulin [2], alpha₁-acid glycoprotein [7] and alpha-fetoprotein [3]. Moreover suppressor macrophages which inhibit an immune response have been found in the spleen cells of experimental animals [7].

This paper described that the immunosuppressive effect of the serum from a dog with squamous cell carcinoma (SC) was detected by the assay of phytohemagglutinin (PHA) induced lymphocyte blastogenesis, and that the abnormal proteins in a tumor-bearing serum resemble a type of immunosuppressive acid protein (IAP) [7, 8] which was found in sera from patients and mice with tumors.

Immunosuppressive effect of the serum

was assayed by the PHA-induced lymphocyte blastogenesis. Normal canine peripheral lymphocytes were prepared by Ficoll-metrizoate (Lymphoprep: Nyegaard, Oslo, Norway) immediately after blood drawing, were washed with RPMI 1640 medium, and then were suspended in RPMI 1640 medium containing PHA-P (10 µg/ml). The suspension of the lymphocytes was divided into two parts. One was cultured with medium containing 10% normal dog serum (NS) and another with containing 10% SC serum (SCS). After the 72 hour incubation, 0.2 µCi of ³H-thymidine (³H-TdR: New England Nuclear, Boston, USA) was added to each culture medium, then the cultures were incubated for additional 24 hours. After incubation, the labeled cells were trapped on glass filter and the ³H-TdR uptake was counted by a liquid scintillation counter. The stimulation index (SI) was expressed as ratio of SCS/NS according to the formula [5].

$$SI = \frac{\text{dpm of } ^3\text{H-TdR incorporated in SCS}}{\text{dpm of } ^3\text{H-TdR incorporated in NS}} \times 100(\%)$$

Table 1. Inhibitory effect of the serum from the dog with squamous cell carcinoma on lymphocyte stimulation

Sera	Mitogen	Incorporation of ³ H-thymidine (mean dpm±SD)	SI ^{a)}
Normal sera	PHA-P	16,792±4,562	100.0
SC serum ^{b)}	PHA-P	3,403± 210	20.2
Normal sera	—	242± 59	—

a) Stimulation index (%).
b) Serum from the dog with squamous cell carcinoma.

The radioactivity of 10⁵ cells was determined in a triplicate test.

The blastogenesis of normal canine lymphocytes stimulated by PHA-P was found in the culture medium containing 10% NS, whereas a definite inhibition of PHA-P response (80%) was found in the culture medium containing 10% SCS as shown in Table 1. The suppressive effect on the blastogenesis of lymphocyte culture *in vitro* might be due to presence of an inhibitory factor in SCS.

For the purpose of finding an immune

Fig. 1. IEF of normal and SC sera.
1: Normal dog serum.
2: Serum from the dog with squamous cell carcinoma (SC).

Fig. 2. Two-dimensional gel electrophoresis of normal and SC sera.
1 and 2: See the remarks of Fig. 1.
SDS-PAGE: SDS-polyacrylamide gel electrophoresis.

suppressive factor in SCS, an analysis of the serum proteins of NS and SCS was performed by the isoelectrofocusing (IEF) ranged pH 2.5 to 5.0 and two-dimensional gel electrophoresis (TDGE). After the run, proteins were stained with 0.066% comassie blue in 20% methanol and 7% acetic acid. IEF gels of NS and SCS stained were illustrated in Fig. 1. Definite difference of protein profiles between NS and SCS was found at acidic region. Acidic specific proteins were found only in SCS but not in NS. Isoelectric points of the acidic proteins were detected around pH 3.2, and these proteins were separated into two protein bands by TDGE as shown in Fig. 2. The molecular weights of two acidic proteins of SCS were calculated to be approximately 57 K and 68 K daltons, respectively.

The electrophoretic properties of acidic proteins detected in the SCS were similar to those of the mouse IAP reported by Shibata *et al.* [7]. The serum from the tumor-bearing dog did not cross-react against the human IAP in the single radial immunodiffusion test (data not shown). The molecular weights of the proteins detected in SCS were 57 K and 68 K by TDGE analysis, whereas the human IAP was a 50 K as reported by Tamura *et al.* [8]. Isoelectric points of the proteins in SCS are similar to those of IAP which were found in human and mouse sera. IAP was detected in the serum of patients with various cancers

and other chronic diseases [9]. In other experiment, we have detected similar acidic proteins in canine sera with chronic diseases. This result suggests that IAP might be common in other animals with various cancer or chronic diseases. Shibata *et al.* [7] indicated that there is a close correlation between the appearance of the suppressor macrophage and the increased IAP level in the serum. The isolation and the characterization of the serum immunosuppressive proteins in the dog with SC are now under investigation.

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要 約

イヌの扁平上皮癌血清中の免疫抑制たん白質 (短報): 桑原正人・田中茂男・小出英興 (日本大学農獣医学部獣医放射線学教室) ——正常リンパ球の phytohemagglutinin に対する反応は, 扁平上皮癌のイヌの血清を添加すると正常なイヌの血清を添加した場合に比べ著しく抑制された。2次元電気泳動法により, この担癌動物の血清中には正常血清では認められない酸性たん白質が検出され, 担癌宿主の免疫機能の抑制に関与していると推定された。