NOTE *Immunology*

High Level Activity of 2', 5'-Oligoadenylate Synthetase in Dog Serum

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ABSTRACT. Most animal cells that are exposed to interferon (IFN) experience an increase in the activity of 2', 5'-oligoadenylate synthetase (OAS), which is an important effector of IFN's antiviral action. OAS activity has been widely used in clinical chemistry as an indicator of IFN activity. In this study, we found that OAS activity in canine serum is 46.0 ± 40.4 nmol/d*l*/hr, which is 10- to 100-fold higher than in other animals such as the cat (1.9 ± 2.1), rabbit (4.0 ± 1.1), and guinea pig (0.3 ± 0.6). The canine OAS protein was detected by Western blotting using a 68M-10 monoclonal anti-murine OAS antibody, and was found to be composed of at least three distinct molecular species of p40 class OAS. Among these, the 40 and 42 kDa components were determined to be the major species in serum and fibroblast cell lines, respectively.

KEY WORDS: canine, 2', 5'-oligoadenylate synthetase, serum.

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Exposure of most animal cells to interferon (IFN) increases the *in vitro* and *in vivo* activity of 2', 5'-oligoadenylate synthetase (OAS), which is an effector of IFN [1–3]. OAS is converted to an active form by binding to double-stranded RNA (dsRNA), which enables it to synthesize ppp(A2'p)nA oligoadenylates (2-5A) from ATP. 2-5A in turn activates a latent endoribonuclease L, which degrades RNA by cleaving at 3'-side of -UpXp- sequences. Therefore the antiviral action of OAS is a result of the inhibition of viral protein synthesis through the degrading action of either viral mRNA or single-stranded genome RNA after activation by viral dsRNA, which is produced as the replication intermediate of genome RNA.

Enzymes other than OAS which catalyze 2', 5'-phosphodiester bond formation between ribonucleotides only after binding dsRNA are rarely found in animal cells, and this has permitted the development of specific and simple OAS activity assays now widely used in clinical chemistry. Changes in OAS activity in leukocytes and sera due to several pathological conditions can be observed [4–8]. This is useful for determining OAS activity in leukocytes or sera during follow-up examinations after intravenous or intramuscular IFN therapy [9–19]. The level of serum OAS activity in humans [16, 18, 19] and mice [20] is reported to be around 0.1 nmol/d*l*/hr as measured using a highly sensitive radioimmunoassay [21]. In this study, we measured the OAS activity in sera from normal dogs and found a 10- to 100-fold higher level of activity compared with other animals. We also detected the canine enzyme by immuno-blot assay using a monoclonal anti-murine OAS antibody [22] .

Eight-week old unvaccinated female beagles were purchased from the Institute for Animal Reproduction (Ibaragi, Japan). Four-month old female beagles which had been vaccinated with attenuated strains of canine distemper virus, canine parvovirus, canine adenovirus type 2, canine parainfluenza virus, and two strains of *Leptospira* bacterins (Vanguard™ 7, Pfizer, Inc., U.S.A.) at the ages of 7- and 13-week old were purchased from Nosan Corporation (Yokohama, Japan). In addition, 6 female beagle dogs and 10 cats which had been kept in our animal houses for more than 1 year were also used. Three-month old SPF rabbits and five-week old guinea pigs were obtained from NISSEIKEN Co., Ltd. (Tokyo, Japan). Sera were prepared in quarantine and measurement of serum OAS activity was carried out by the DEAE method [23]. In brief, oligomerized products derived from in the presence of dsRNA (polyriboinosinic acid: polyribocytidilic acid (pIC), Amersham Biotech., U.S.A.) from ^{3}H-ATP (American Radiolabeled Chemicals, Inc., U.S.A.) were separated by DEAE cellulose chromatography. The activity was expressed in units of nmol ATP converted to oligomer per d*l* of serum per hour of incubation time (nmol/d*l*/hr).

OAS activity was measured for a total of 18 dogs (Fig. 1). They included six 8-week old unvaccinated female beagles (Group A), six 4-month old double vaccinated female dogs with twice vaccination (Group B), and six adult dogs older than 1 year (Group C). OAS activity in the dogs was significantly higher than that found in the serum of the cats, rabbits, and guinea pigs. In particular, dogs from Group A showed the highest activity, although it varied somewhat individually. There seemed to be correlation between OAS activity and age, and influence on activity due to prior vaccination with live attenuated viruses or bacterins. Systematic survey is necessary to address detailed characterization on variety in dog species or physiological changes of serum OAS activity. On average, OAS activity in the dogs was 46.0 ± 40.4 nmol/d*l*/hr, which was higher than the activities found in the cats (1.9 ± 2.1), rabbits (4.0 ± 1.1), and guinea pigs (0.3 ± 0.6). OAS activity in normal animals ranged near the detection limit of the assay system employed in this study. Even after infection with viruses or bacteria, OAS activity increases only 10-fold [16, 18, 19]. After systemic

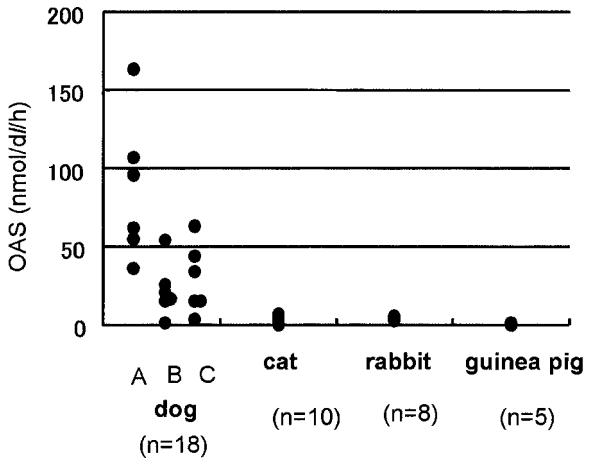

Fig. 1. OAS activity in the sera of dogs, cats, rabbits and guinea pigs. OAS activity was assayed using 50 μl of serum by the DEAE method [23]. One circle represents one animal. Among the dogs, three groups were examined: Group A, 6 8-week old unvaccinated female beagles; Group B, 6 4-month old vaccinated female beagles; and Group C, 6 adult dogs older than 1 year.

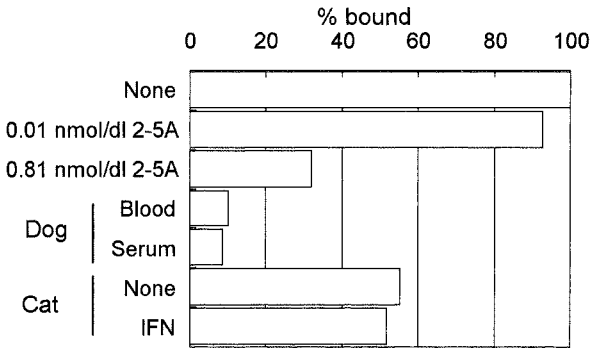

Fig. 2. Identification of 2-5A molecule by competitive RIA. Canine serum and blood from non-immunized dogs, and cat sera before and 8 hr after subcutaneous administration of 10^6 units/kg of feline IFN-ω were tested using the RIA kit [21] according to the manufacturer's protocol. The competition assay was also carried out using authentic 2-5A. The highest and lowest competition levels are also indicated, which were obtained using 0.81 nmol/d*l* or 0.1 nmol/d*l* of 2-5A, respectively.

administration of IFN in man [8–14, 16–19], mouse [20] and calf [15], OAS activity was within the range of a radioimmunoassay (RIA) kit (0.01–0.81 nmol/d*l*/hr, Eiken Chemical Co., Ltd., Tokyo, Japan [21]), which have been widely used in these experiments. On the other hand, most of OAS activity found in normal female beagles were higher than upper limit of this kit.

The DEAE method measures pIC-dependent formation of oligonucleotides from radio-labeled ATP. To confirm that the oligonucleotide samples contained 2-5A molecules, we tested whether the reaction products could compete with radio-labeled authentic 2-5A for epitope binding sites in anti-2-5A antiserum using the RIA kit. The unlabeled 2-5A products inhibited binding of ^{125}I-labeled 2-5A molecules to the antibody in a dose-dependent manner (Fig. 2). The reaction products in dog sera completely inhibited 2-5A binding to the antibody. Essentially the same result was obtained with whole blood, which indicated that OAS was not secreted blood cells after clotting. In contrast, competitive inhibition of binding to the antibody was observed with 2-5A products from cat sera (Fig. 2). OAS activities in the cat, used in this experiment, were 0.32 nmol/d*l*/hr and 0.38 nmol/d*l*/hr, before and 8 hr after subcutaneous administration of 10^6 units/kg feline IFN-ω (Kyoritu Seiyaku Co., Ltd., Tokyo, Japan), respectively.

We next examined the OAS protein by Western blotting using a monoclonal antibody against mouse OAS (68M-10 mAb, [22]). The reactivity of anti-murine OAS antibody against canine OAS was tested using virus-infected cell cultures. For this purpose, canine A-72 cells (ATCC No. CRL-1542, [24]) were grown in 20% FBS L15 medium and canine MDCK cells (ATCC No. CCL-34) were grown in 5% FBS DMEM. Canine D-17 cells (ATCC No. CCL-183, [26]), canine Cf2Th (ATCC No. CRL-1430), human HeLaS3 cells (ATCC No. CCL-2.2), and murine L929 cells (ATCC No. CCL-1) were grown in 10% FBS DMEM. To activate IFN and OAS, these cells were treated for 24 hr with Newcastle disease virus (NDV, 10^8 EID_{50}/m*l*, NISSEIKEN Co., Ltd.) which was inactivated by UV irradiation. Figure 3 shows OAS activity in 50 μg of cell lysate. OAS activity in Cf2Th, A72, MDCK, L929 and HeLa S3 cells was relatively low prior to virus infection and increased after NDV treatment. In contrast, D-17 cells showed high constitutive levels of OAS activity. The D-17 cell line was established from a lung metastasis of an osteosarcoma in a poodle as non-producer of type C retroviruses [25]. Low IFN activity was detected in the supernatant of the D-17 cell cultures even though there was no induction stimulus (data not shown).

Western blotting was performed on all samples according to a previously published procedure [22, 23]. Immuno-reactive bands were detected only after NDV infection in all cell lines except for the A72 and HeLaS3. The major species of OAS protein was estimated to be either 42 kDa (D-17, Cf2Th, L929 cells) or 40 kDa (MDCK) based on their respective rate of migration. For these cases, their band intensities correlated well with their respective OAS activity. The same result was obtained after the cells were treated with 10 μg/m*l* pIC plus 100 μg/m*l* DEAE dextran (Pharmacia Biotech., Inc., U.S.A.), which together was used as an IFN inducer (data not shown). The failure to detect OAS in HeLaS3 cells was due to a lack of affinity of the 68M-10 mAb for human OAS. A72 cells possessed little OAS activity and OAS protein after infection with inactivated NDV (see Fig. 3). However, OAS activity increased in tandem with an increase in OAS protein level when A72 cells were exposed to canine IFN (Fig. 4, [26]).

A detailed characterization of the OAS protein in dog

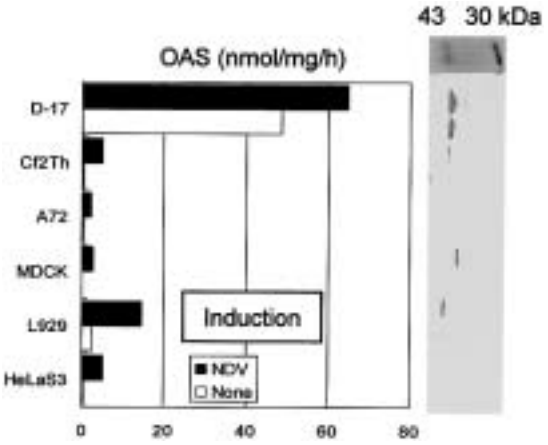

Fig. 3. Detection of the canine OAS proteins by immunoblotting using monoclonal anti-murine OAS antibody. Canine, mouse and human cell lines were treated with UV-inactivated NDV for 24 hr. OAS activity in 50 μg cell lysates was measured by the DEAE method [23]. In parallel, 5 μg aliquots of lysate were subjected to Western blotting using 68M-10 mAb [22]. The bands were visualized with diaminobenzidine and H_2O_2.

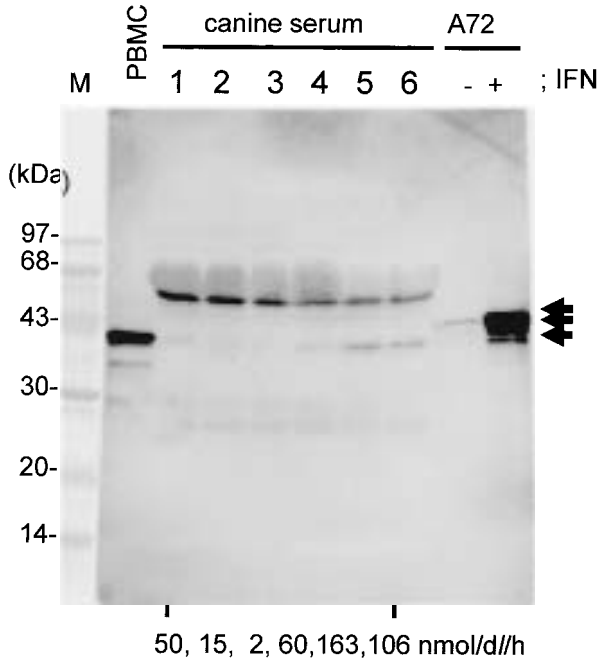

Fig. 4. Western blotting analysis of the canine OAS. One μl aliquot each of the sera from all groups was analyzed by Western blotting using 68M-10 mAb [22], and the bands were visualized with a chemi-luminescent kit and CCD camera imaging system. As controls, cell lysates from A72 cells treated with or without 10^3 LU/m*l* IFN-β, and from peripheral blood mononuclear cells prepared from a dog were analyzed. OAS activity of each canine serum is indicated below the lane. The three species of p40 class OAS detected in A72 cells are indicated by arrows on the right.

sera was obtained from Western blots that were stained using highly sensitive chemi-luminescent reagents (ECL Plus, Amersham Pharmacia Biotech., U.S.A.) and detected with a photomultiplier light detection system (LAS-1000; Fuji Photo Film Co., Ltd., Tokyo, Japan). Three species of OAS with apparent molecular sizes of 42, 41 and 40 kDa were detected for canine A72 cells treated with 10^3 LU/m*l* of canine IFN-β (Fig. 4, A72 lanes). The 42 kDa component was the major species among the three forms of OAS. Also, the 40 kDa component was only detected in sera as well as peripheral blood mononuclear cells (PBMC) (Fig. 4). Two other bands of about 50 and 30 kDa were detected in canine sera, and they corresponded to canine immunoglobulins which reacted with secondary peroxidase-conjugated anti-murine immunoglobulin G antibody.

Three forms of OAS protein (p100, p70, p40) have been characterized in human and murine cells [27] in concert with the multigenic nature of OAS in humans [27] and mice [28, 29]. A single p40 class OAS gene has been identified in humans while 9 genes have been detected in mice [27–29]. Even though the organization of OAS genes has not been determined for dogs, we detected three species of p40 class canine OAS in canine sera (see Fig. 4). OAS protein synthesis and activity was usually induced intracellularly by the action of IFN. The very low level of OAS activity detected in animal serum may have been due to the release of OAS protien from some cells. However, the exact origin remains to be elucidated. This is the first report about the detection of OAS protein in animal serum. Since PBMC produce the 40 kDa species of OAS protein, they are one origin of serum OAS.

At present, it is still unclear as to why a high level of OAS exists in dog serum. Apparently neither dog age nor vaccination history influence the OAS level (see Fig. 1). The expression of OAS has been demonstrated immunohistochemically for the brain [30] and digestive tract [31] in the mouse, as well as for chicken erythrocytes [32, 33]. The dogs (female beagles, at least) possess high levels of spontaneously induced OAS activity. However, we can not exclude the possibility that the dogs used in this study were persistently infected with viruses without exhibiting any clinical signs.

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