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CHANGES IN SERUM C-REACTIVE PROTEIN LEVELS IN DOGS WITH VARIOUS DISORDERS AND SURGICAL TRAUMAS

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ABSTRACT

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The serum levels of C-reactive protein (CRP) produced as an inflammatory response in dogs with various disorders and surgical traumas were measured by enzyme-linked immunoabsorbent assay and slide reversed passive latex agglutination test (RPLA). The CRP levels were greatly increased 1–2 days after surgery in most of the dogs ($n=29$) subjected to surgery. These levels had markedly decreased by the time the sutures were removed. In dogs with various disorders ($n=58$), the serum CRP levels at first diagnosis were high in infectious diseases. In dogs from which paired serum samples were examined, the serum CRP usually showed a decrease with improvement in the condition ($n=11$) or a terminal increase ($n=4$) but, conversely, some showed an increase with improvement in the condition ($n=3$).

Keywords: acute phase proteins, dogs, C-reactive protein, enzyme-linked immunoabsorbent assay, inflammation, serum, surgery

Abbreviations: CRP, C-reactive protein; ELISA, enzyme-linked immunoabsorbent assay; Latex, soap free latex; RPLA, reversed passive latex agglutination

INTRODUCTION

C-reactive protein (CRP) is one of the acute-phase proteins that are produced as an inflammatory reaction in man following tissue damage caused by inflammation, infection or injury (McFarlane *et al.*, 1967; Sabel and Hanson, 1974). Interleukin-6 is considered to be a major inducer of CRP production (Gauldie *et al.*, 1987; Nijsten *et al.*, 1987). CRP is a typical acute-phase protein whose behaviour has been well studied in diseases (Pepys and Baltz, 1983; Maury, 1985).

Since the first detection of CRP in the dog (Caspi *et al.*, 1984), methods for quantifying its serum concentration have been introduced and characterization of its behaviour has been attempted (Caspi *et al.*, 1987; Conner *et al.*, 1988; Ndung'u *et al.*, 1991). In human medicine, CRP has been recognized as the most stable indicator of inflammation compared to fever, white blood cell count and erythrocyte

sedimentation rate, etc., and changes in serum CRP are assayed for evaluation of pathological conditions (Mallya *et al.*, 1982; Peltola and Rasanen, 1982; Whicher *et al.*, 1985). However, individual acute-phase proteins showed different time courses of incremental response (Kushner, 1982). It is, therefore, necessary to determine the species-specific responsiveness of acute-phase proteins. The behaviour of serum CRP in the dog is not well known.

The prime purpose of this study was to quantify the serum CRP levels in dogs with various disorders and surgical traumas by enzyme-linked immunoabsorbent assay for comparison with the levels resulting from other types of acute-phase reaction. Semiquantification of the serum CRP levels in these dogs was also carried out by a simple and rapid slide reversed passive latex agglutination test to assess the practical applicability of this method.

MATERIALS AND METHODS

Dogs and sera

The serum CRP levels were measured in sera from 153 household dogs. Complete blood cell counts and routine chemical analyses were performed on all blood samples collected. Blood was collected at the time of first diagnosis from 66 normal dogs and from 29 dogs that were scheduled for surgery, at 1–2 days after surgery and again at the time of suture removal, 8 days postoperatively. Blood was also collected at first diagnosis from 58 dogs with various disorders and again whenever a clinically apparent change was detected during the course of treatment. Sera collected 24–48 h after experimental surgery from 20 dogs of various breeds were pooled for purification of canine CRP. All serum samples were stored at −80°C until ready for use.

Clinical cases

Surgical

Sera from dogs subjected to the following procedures were tested: oophorohysterectomy due to pyometra ($n=3$), oophorohysterectomy for contraception ($n=3$), orthopaedic surgery ($n=4$), tooth extraction ($n=4$), and excision of superficial tumours ($n=5$).

Disorders for which surgery was not indicated

Dogs with various disorders consisted of 4 with bacterial enteritis, 2 with leptospirosis, 4 with parvovirus infection, 17 with a variety of tumours, 4 with haemorrhagic enteritis, 2 with anal polyps, 4 with odontolithiasis and gingivitis, 8 with various dermal diseases, 6 with gastroenteritis, 3 with eye diseases, 2 with rickets and 2 with nephritis. Viral and bacterial infections were diagnosed from the clinical impression but isolation of viruses and bacteria was not carried out.

Enzyme-linked immunoabsorbent assay (ELISA)

Purification of canine CRP from pooled serum, preparation of rabbit anti-canine CRP serum and ELISA were performed as previously described (Yamamoto *et al.*, 1992). A serum containing 444 μg/ml of CRP was used as the reference serum for ELISA. The lowest detectable limit of canine serum CRP by ELISA was 3.5 ng/ml.

Slide reversed passive latex agglutination (RPLA)

Soap-free latex (latex) (Sekisui Chemical Co., Ltd, Osaka, Japan) having a grain size of 0.12 μm was used to prepare IgG antibody-sensitized latex. IgG antibody, isolated from the rabbit anti-canine CRP serum on a protein G–Sepharose 4B column (Pharmacia LKB Biotechnology, Uppsala, Sweden) (Åkerström and Björck, 1986), was used at 75 μg/mg to sensitize the latex.

For the slide RPLA, 20 μl of canine serum diluted serially with 0.01 mol/L phosphate-buffered saline (PBS, pH 7.2) was mixed with an equal volume of the IgG antibody-sensitized latex on a black glass slide and the agglutination of the latex was assessed after 3 min. Using the same serum that was used as the reference in the ELISA, the concentration of CRP detectable by this slide RPLA was found to be 0.43 μg/ml. For semiquantitative determination in μg/ml of the CRP concentrations in the serum samples, the maximum dilution of the serum at which latex particles agglutinated was multiplied by 0.43.

RESULTS

Normal dogs

The serum CRP levels ranged from 2.40 to 30.04 μg/ml (8.43 ± 4.86 μg/ml; mean ± SD) by ELISA and from 2.20 to 17.20 μg/ml (8.52 ± 6.25 μg/ml) by RPLA.

Dogs subjected to surgery

The serum CRP levels in these dogs were, at the time of first diagnosis, slightly higher than the normal level except in those scheduled for contraceptive operation (Table I). The serum CRP levels were markedly increased 1–2 days after surgery in all the dogs except those that had undergone tooth extraction. The postoperative course was quite uneventful in all these dogs. The serum CRP levels were markedly lower 8 days after surgery when the sutures were extracted. In the dogs with pyometra and in those with superficial tumours, the serum CRP levels were by then below the preoperative levels (Figure 1). The mean serum CRP levels following surgery, with the incremental ratios as compared to the normal level, were 383.2 μg/ml (45-fold) in the dogs subjected to orthopaedic surgery, 249.2 μg/ml (29-fold) in those subjected to excision of superficial tumours, 181.6 μg/ml (21-fold) in those with pyometra subjected to oophorohysterectomy, and 136.2 μg/ml (16-fold) in those subjected to contraceptive operation.

TABLE I
Results from ELISA for canine serum CRP and WBC counts in surgically treated dogs

Surgery	No. of dogs	Median (range) for serum CRP (μg/ml) and WBC ($\times 10^9$/L)					
		At first diagnosis		After surgery		At suture removal	
		CRP	WBC	CRP	WBC	CRP	WBC
Oophorohysterectomy Pyometra	3	61.4 (15.3–107.5)	0.016 (0.014–0.034)	181.6 (22.0–269.8)	0.035 (0.016–0.045)	19.1 (13.3–171.4)	0.013 (0.013–0.030)
Contraception	3	11.7 (7.1–15.2)	0.009 (0.005–0.013)	136.2 (86.1–234.9)	0.020 (0.011–0.027)	36.0 (19.5–48.8)	0.018 (0.012–0.021)
Orthopaedic surgery	4	47.6 (15.2–127.6)	0.018 (0.002–0.029)	383.2 (244.5–558.9)	0.018 (0.014–0.037)	72.3 (46.6–124.7)	0.024 (0.011–0.040)
Tooth extraction	4	30.5 (10.7–49.2)	0.018 (0.006–0.032)	61.2 (30.6–91.8)	0.018 (0.008–0.033)	72.5 (5.7–48.2)	0.018 (0.007–0.037)
Excision of superficial tumours	15	78.5 (5.1–307.1)	0.017 (0.008–0.041	249.2 (174.0–364.7)	0.020 (0.011–0.049)	39.8 (15.6–266.3)	0.018 (0.007–0.026)

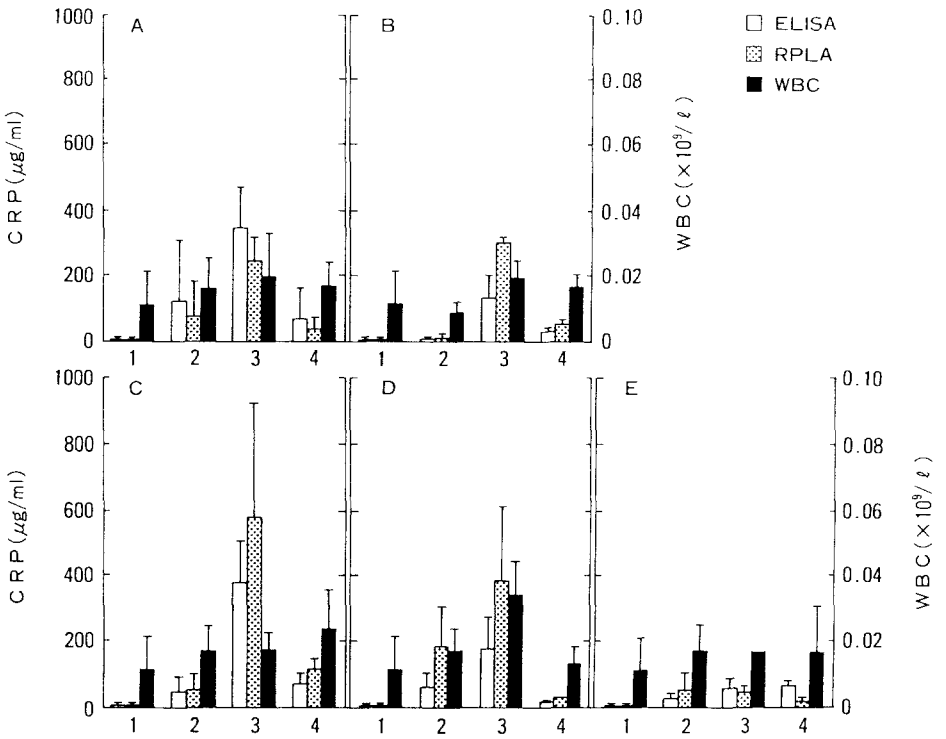

Figure 1. Changes in serum CRP (mean ± SEM) and WBC (mean ± SEM) in 29 dogs. A, oophorohysterectomy due to pyometra ($n=3$); B, oophorohysterectomy for contraception ($n=3$); C, orthopaedic surgery ($n=4$); D, excision of superficial tumours ($n=15$); E, tooth extraction ($n=4$). 1, normal CRP level (control); 2, at first diagnosis; 3, 1–2 days after surgery; 4, at suture removal

Dogs with various disorders

Marked differences in concentrations of serum CRP were found at first diagnosis depending on the type of disease. In all the dogs with infections (leptospirosis, parvovirus infection and bacterial enteritis) and in some of those with superficial tumours or haemorrhagic enteritis, the serum CRP levels were markedly elevated and were almost as high as those seen following surgery. In dogs with dermal diseases and gingivitis, the serum CRP levels were at most only slightly increased. In dogs with gastroenteritis, eye diseases, rickets or nephritis, increases in serum CRP could hardly be confirmed (Table II). In the 11 dogs that had elevated serum CRP levels at first diagnosis, the levels were markedly decreased when the clinical signs had improved at convalescence 2–26 days after treatment and were lower than those found at first diagnosis (Figure 2). In the dogs with gastroenteritis ($n=3$), the serum CRP levels at first diagnosis were slightly higher than the normal value but the WBC count was markedly greater. The WBC count returned almost to the normal value during

convalescence 3–8 days after treatment, but the serum CRP levels had by then increased to approximately seven times those at first diagnosis (Figure 2). Four dogs had only slightly elevated serum CRP levels at first diagnosis but died during treatment. The serum CRP levels in these dogs were markedly increased just before death, 5–14 days after treatment (Figure 2).

Semiquantitative determination of serum CRP concentrations by RPLA

The RPLA values tended to be higher than the ELISA values. It was, however, confirmed that the semiquantitative RPLA values changed in parallel with the quantitative ELISA values (Figures 1 and 2).

DISCUSSION

Increases in canine CRP as an inflammatory reaction to surgery (Caspi *et al.*, 1984; Conner *et al.*, 1988) and to infection or some diseases (Caspi *et al.*, 1987; Ndung'u *et al.*, 1991) have previously only been shown by semiquantitative assay. In this study, the changes in serum CRP levels in the dogs with surgical traumas and various disorders were confirmed by quantitative immunoassay to further characterize the behaviour of canine CRP.

CRP rapidly increases following acute stimulation and the acute-phase response occurs more rapidly in dogs than in man. Of all acute-phase proteins, CRP has been found to respond mostly rapidly to surgery, with a high rate of increase in the dog (Conner *et al.*, 1988). The results obtained in this study confirmed that the increase in serum CRP in the dogs subjected to surgery was generally related to the intensity of the surgical trauma. The CRP increase was larger when more severe tissue injury was produced in muscle or bone marrow by procedures such as orthopaedic surgery. In many of the dogs subjected to surgery, the WBC count, which had increased following surgery, was still elevated when the sutures were removed, by which time the clinical signs had undoubtedly improved. The changes in CRP level and WBC count in the dog do not seem to have been compared previously, but the results of this study suggest that CRP is more useful than the WBC count for assessing the severity of inflammation. Thus, variation in the WBC count may not serve as a reliable indicator of inflammation in the dog any more than in man (Fischer *et al.*, 1976). The fact that the canine CRP, which had been elevated, was markedly reduced by the time of suture removal and following abatement of the clinical signs, seems to suggest that the half-life of canine CRP is short, as is that of lapine CRP (Chelladurai *et al.*, 1982). If so, the short half-life may render CRP a convenient tool for estimating the improvement of inflammation in veterinary clinical medicine.

Serum CRP responses in dogs with various disorders have only been reported by Caspi *et al.* (1987) using single serum samples. They found that the serum CRP levels in their dogs were elevated to different degrees by different diseases and that, in all types of disorder, there were nonresponsive dogs that had CRP levels <0.5 μg/ml, which was below the limit of detection by their semiquantitative assay. Although the number of dogs was limited, it was confirmed that the elevation of serum CRP at first diagnosis differed considerably with the type of stimulation. In examining paired

TABLE II
Results from ELISA for canine serum CRP and WBC counts in dogs with various disorders when first diagnosed

Diseases	No. of dogs	Serum CRP (μg/ml)		WBC ($\times 10^9$/L)	
		Range	Median	Range	Median
Marked CRP increases					
Leptospirosis	2	139.3 – 393.6	266.4	0.018 – 0.022	0.020
Bacterial enteritis	4	124.7 – 228.2	169.3	0.014 – 0.026	0.020
Parvovirus infection	4	125.8 – 237.8	165.9	0.002 – 0.004	0.002
Tumour	17	4.9 – 231.1	66.3	0.008 – 0.043	0.021
Haemorrhagic enteritis	4	6.2 – 111.8	56.6	0.009 – 0.017	0.013
Slight CRP increases					
Polyp	2	15.3 – 50.6	32.9	0.010 – 0.034	0.022
Odontolithiasis and gingivitis	4	4.2 – 49.4	28.9	0.004 – 0.032	0.013
Dermatitis	8	2.4 – 94.1	26.3	0.008 – 0.014	0.013
No CRP increases					
Gastroenteritis	6	6.5 – 33.3	17.6	0.013 – 0.065	0.025
Eye disease	3	4.9 – 11.4	8.0	0.010 – 0.022	0.015
Rickets	2	7.0 – 8.1	7.5	0.014 – 0.022	0.018
Nephritis	2	4.1 – 9.6	6.9	0.007 – 0.010	0.009

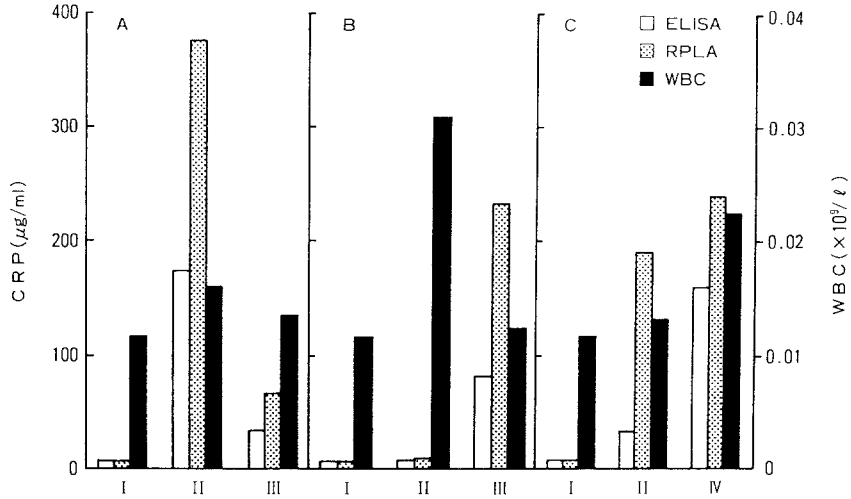

Figure 2. Changes in serum CRP (mean) and WBC (mean) at first diagnosis and after treatment in dogs with various disorders. A, cases ($n=11$) with decreased serum CRP levels following improvement of the condition by treatment; B, cases ($n=3$) with increased serum CRP levels following improvement of the condition by treatment; C, cases ($n=4$) that died after treatment. I, normal serum CRP level (control); II, at first diagnosis; III, at convalescent stage; IV, at death

serum samples, it was found that the serum CRP levels increased despite the improvement of the clinical signs after treatment in some of the dogs that did not have elevated serum CRP levels at first diagnosis. This may indicate that, following recovery from the primary disease, some secondary conditions responsible for the CRP increase might have taken place. Further detailed studies on the variations of serum CRP level in a wider variety of diseases are necessary.

CRP is known to increase 500- to 1000-fold from its baseline level in man in response to acute inflammation (Kushner, 1982). Such a dramatic increase has not been documented in the dog. Though not shown in this paper, the greatest concentration of CRP that we have observed was a serum with 981.6 μg/ml (115 times the normal level). The higher baseline levels for serum CRP in healthy household dogs than in man (Claus *et al.*, 1976) or in Beagle dogs reared in clean experimental animal laboratories (Yamamoto *et al.*, 1992) may account for this relatively low increment in CRP.

Unless other simple methods of quantification, such as immunoturbidimetric assay (Eckersall *et al.*, 1991), are more easily used and/or more accurate in the measurement of canine CRP, the rapid procedure of RPLA would be a useful method of semiquantitative immunoassay.

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