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Determination of C-reactive protein by turbidimetric immunoassay (TIA) in sheep

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ABSTRACT

C-reactive protein (CRP) has been shown to be an important part of acute phase reaction in domestic mammals. We investigated the presence of CRP in Slovenian sheep in order to obtain a better understanding of the distribution of CRP in ovine species. The reference sample comprised fifty-two clinically healthy female sheep (14 Solča breed and 38 Bovško breed, aged between six months and four years). Assay for CRP was carried out in blood serum using a turbidimetric immunoassay (TIA) with rabbit polyclonal antibodies against human CRP molecule. Values for CRP ranged between 0.000 and 13.600 mg/l, while concentration of CRP (overall mean±s.e.m.) was 6.280±0.429 mg/l. Sample reference limits (95% confidence interval) were between 0.086 and 12.474 mg/l. Mean CRP concentrations for both breeds were 5.235±0.429 for Bovško sheep and 9.115±0.647 mg/l for Solča sheep, respectively ($P<0.001$). It can be concluded that CRP serum levels represent the result of compromised metabolic and immunological activity. This study was thought to be helpful in distinguishing between normal and pathological serum values for CRP in sheep.

Key words: turbidimetric immunoassay, C-reactive protein, reference limits, sheep

Introduction

The acute phase reaction is a very old element of natural immunity. Besides non-immune factors, the natural killer cells, T- and B-lymphocytes, as well as some serum proteins, are able to recognize antigene epitopes and activate the immune system. A sudden rise in cytokines, especially IL-1, IL-6 and TNF alpha, is followed by production of several acute phase proteins such as fibrinogen, haptoglobin, serum

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amyloid, ceruloplasmin and C-reactive protein, the concentrations of which increase up to 1,000-fold (reviewed by BERCZI et al., 1998).

Seventy years ago, C-reactive protein (CRP) was the first acute phase protein determined in serum from humans with *Pneumococci* infection. Until now the chemical structure of CRP has been well recognized. The CRP protein belongs to pentraxins (BOTTAZZI et al., 1997). Pentraxins are a family of pentameric proteins, examined by electron microscopy forming typically pentameric discoid subunits (SHRIVE et al., 1996) with a molecular mass of 20,000 Daltons each. CRP was named according to its *in vitro* ability to bind to the C-polysaccharide of *Streptococci* bacteria (BEAUFORT et al., 1997). The *in vivo* functions of CRP still remain unclear. According to *in vitro* experiments it can be concluded that these functions include the ability to activate complement cascade by classical pathway (ABERNATHY et al., 1996; WOLBINK et al., 1996). Other functions assigned to CRP are modulation of activity of blood platelets (CHERYK et al., 1996), erythrocyte aggregation (WENG et al., 1996), opsonization of killed (but not live) bacteria (BEAUFORT et al., 1997), and opsonization of necrotic tissue and cellular debris (ECKERSALL and CONNER, 1988). All CRP's studied have the capacity for binding phosphorylcholyn that is mediated through calcium ions (NORDIN et al., 1996; SHRIVE et al., 1996). However, some differences among domestic species are recognized. For example, unlike human CRP, which is not glycoprotein, canine CRP is glycolsylated on two of five pentamer subunits (ECKERSALL and CONNER, 1988). Human CRP agglutinates lecithin micelles, while bovine CRP cannot because of its low affinity to the phosphorylcholine (MORIMATSU et al., 1991). Although MORIMATSU et al. (1991) expressed some reservations about the use of antibodies on human to bovine CRP, there is a strong similarity of amino acids composition and pentameric configuration for both pentraxins. Moreover, CHERYK et al. (1996) make the point that anti-human CRP antibodies are cross-reactive with bovine CRP.

As recently reported (ZHANG et al., 1996), signal transducers and activators of transcriptions (STAT3) family members are mediators of IL-6 induced CRP gene activation. There is only one gene in the higher vertebrates that controls synthesis of CRP protein in the liver. In contrast, animal species whose immune system operates without immunoglobulins produce more than one CRP with similar functions but individual specifity (LIU et al., 1987). Hence, no polymorphism or deficiency state for CRP in humans is known. The sequence homology of CRP gene organisation indicates that they arose from an ancestral gene that has been highly

conserved throughout 500 million years of evolution (LIU et al., 1987; LUND and OLAFSEN, 1998).

Studies in several animal species provide evidence that the C-reactive protein plays an important role in physiology research and diagnostics (BURTON et al., 1994; TOUSSAINT et al., 1995; ALAVA et al., 1997; ZIMMERMANN et al., 1998). Until now, the role of CRP in sheep has not been well elucidated. Hence, we investigated the presence of CRP in a Slovenian sheep population in order to obtain a better understanding of the distribution of CRP in ovine species.

Materials and methods

Experimental animals comprised fifty-two female sheep, clinically healthy, aged between six months and four years, and were drawn from two of the dominant Slovenian sheep population. The first (N=38) was the Bovško breed, which is a small sheep from the Alpine region. The second, (N=14) Solča breed, is the Slovenian breed most frequently reared in all of the Slovenian regions. During the experiment the sheep were stall housed. Hay, grass silage and vitamin-mineral mix were offered regularly.

Blood samples were collected by jugular venipuncture into the Vacuette system (Greiner, Austria) with a clot activator. Serum CRP concentrations were determined by turbidimetric immunoassay (TIA) using liquid phase immunoprecipitation reaction with polyclonal antibodies obtained by rabbit immunisation with human CRP (Protiline, BioMerieux). The formation of insoluble antigen-antibody complexes was monitored at 340 nm by the increase in turbidity, which is proportional to the concentration of CRP in the sample. Analytical performances of TIA concerning human CRP were 2.5% and 4.0% for intra-series and inter-series variation, respectively, at medium levels of CRP. An automatic centrifugal analyser (Hycell, France) was used in this experiment.

With the aim of establishing the reference range for CRP, the data from the reference sample were fitted to a Normal (Gaussian) distribution. The goodness-of-fit was checked using Kolmogorov-Smirnov test, according to the IFCC reference method and flow charts (JONES and PAYNE, 1997). To establish a reference interval we included the central 95% fraction of the reference distribution (mean±2s.d.). The difference between groups was tested using Student's T-test.

Results

The overall mean±SEM concentration of CRP for experimental sheep, irrespective of breed, was 6.280±0.429 mg/l. Minimal and maximal values measured were between 0.000 and 13.600 mg/l of serum, respectively. Data for both breeds indicates that it matches the pattern anticipated to be drawn from a population with a normal distribution (P= 0.227). The estimated lower and upper values for overall mean ranged between 5.480 and 7.142 mg/l of CRP at the 95 % confidence level. The reference limits for CRP between 0.086 and 12.474 mg/l were at the same level.

Table 1. Descriptive statistics and comparison of CRP values for sheep breeds. Serum CRP concentrations were determined by turbidimetric immunoassay (TIA) using liquid phase immunoprecipitation reaction with polyclonal antibodies obtained by rabbit immunisation with human CRP (Protiline, BioMerieux)

CRP mg/l	Bovško sheep	Solča sheep
N	38	14
Minimum	0.000	5.270
Maximum	13.000	13.600
Mean	5.235***	9.115***
SD	2.645	2.241
95% CI for mean	4.366-6.104	7.717-10.513

***P<0.001; CI=confidence interval

The results for a particular breed were substantially different. The mean CRP concentrations were 5.235±0.429 for Bovško sheep and 9.115±0.647 mg/l for Solča sheep, respectively. The difference in the means (t value= 4.794) of these two groups was significant (P<0.001).

Discussion

The present study confirms that ovine CRP cross-react with polyclonal rabbit antibodies against human CRP. The results of this experiments also showed the potential (although non-specific) of TIA in elucidation of physiological changes and their possible relationship to between breed variations. The reference range was assessed on a sufficient number of animals according to IFCC flow chart (JONES and PAYNE,

1997). Thus, our results are useful for specific diagnostic procedures in animals with symptoms of diseases.

The differences observed between breeds were unexpected, due to strong genetic homogeneity for CRP protein among species and within species, also (LIU et al., 1987; LUND and OLAFSEN, 1998). Thus, the genetic background of these two breeds is somewhat different. The wild ancestor of Solča sheep is *Ovis vignei* var. *arkal*, and of Bovško sheep *Ovis ammon* var. *mussimon*.

It seems reasonable to postulate that some other reasons are responsible for such a difference. Using artificial selection in sheep breeding, the frequencies of particular genes were modulated, especially from an economical point of view. With every new generation the gene pool is removed from its natural relations and from its biological optimum at the same time. In general, this deviation reflects itself through different endocrinological, immunological and metabolic shifts, as suggested by ELSASSER et al. (1997) and NONNECKE et al. (1997). Until now, selection-dependent changes concerning immunological fitness of the sheep population have not been documented. The different CRP levels observed can be attributed to the differences in terms of productivity between breeds. The Bovško sheep is a milk-purpose breed, whereas the Solča sheep is a meat-wool purpose breed. In agreement with this hypothesis, MÜLLER et al. (1998) found a significant correlation between CRP and thyroxin ($r=0.35$) and CRP and cholesterol concentrations ($r=0.52$) in breeding bulls. If we advance this concept to ovine species it can be concluded that CRP serum levels represents the result of compromised metabolic and immunological activity. Very similar effects have previously been seen in dairy cows (MORIMATSU et al., 1991; ZIMMERMMAN et al., 1998).

This study contributes to a better understanding of the distribution of CRP in ovine species. The reference values presented here could be useful in physiological research and diagnostics. The question that remains is this: what if the CRP concentrations are elevated through disease or other tissue damage. To answer this, further research is needed.

References

ABERNATHY, V. J., R. O. WEBSTER, T. E. DAHMS (1996): C-reactive protein inhibits increased pulmonary vascular permeability induced by FMPL in isolated rabbit lungs. Am. J. Physiol. 40, H507-H513.

ALAVA, M. A., N. GONZALES-RAMON, P. HEEGAARD, S. GUZYLACK, M. J. M. TOUSSAINT, C. LIPPERHEIDE, F. MADEC, E. GRUYS, P. D. ECKERSALL, F. LAMPREAVE, A. PINEIRO (1997): Pig-MAP, porcine acute phase proteins and standardisation of assays in Europe. Comp. Haematol. Int. 7, 208-213.

BEAUFORT, DE A. J., J. A. M. LANGERMANS, A. M. MATZE-VAN DER LANS, P. S. HIEMSTRA, J. M. VOSSEN, R. VAN FURTH (1997): Difference in binding of killed and live *Streptococcus pneumoniae* serotypes by C-reactive protein. Scand. J. Immunol. 46, 597-600.

BERCZI, I., D. A. CHOW, E. R. SABBADINI (1998): Neuroimmunoregulation and natural immunity. Dom. Anim. Endocrinol. 15, 273-281.

BOTTAZZI, B., V. VOURET-CRAVIARI, A. BASTONE, L. DE GIOLA, C. MATTEUCCI, G. PERI, F. SPREAFICO, M. PAUSA, C. D'ETTORRE, E. GIANAZZA, A. TAGLIABUE, M. SALMONA, F. TEDESCO, M. INTRONA, A. MANTOVANI (1997): Multimer formation and ligand recognition by the long pentraxin PTX3 - Similarities and differences with the short pentraxins C-reactive protein and serum amyloid P component. J. Biol. Chem. 272, 32817-32823.

BURTON, S. A., D. J. HONOR, A. L. MACKENZIE, P. D. ECKERSAL, R. J. MARKHAM, B. S. HORNEY (1994): C-reactive protein concentration in dogs with inflammatory leukograms. Am. J. Vet. Res. 55, 613-618

CHERYK, L. A., M. A. HAYES, P. A. GENTRY (1996): Modulation of bovine platelet function by C-reactive protein. Vet. Immunol. Immunopathol. 52, 27-36.

ECKERSALL, P. D., J. G. CONNER (1988): Bovine and canine acute phase proteins. Vet. Res. Comm. 12, 169-178.

ELSASSER, T. H., S. KAHL, N. C. STEELE, T. S. RUMSEY (1997): Nutritional modulation of somatotropic axis-cytokine relationships in cattle: a brief review. Comp. Biochem. Physiol. A116, 209-221.

JONES, R. G., R. B. PAYNE (1997): Clinical Investigation and Statistics in Laboratory Medicine. ABC Venture Publ. London.

LIU, T. Y., C. SYIN, N. Y. NGUYEN, A. SUZUKI, R. A. BOYKINS, K. J. LEI, N. GOLDMAN (1987): Comparison of protein structure and genomic structure of human, rabbit, and Limulus C-reactive proteins: possible implications for function and evolution. J. Protein Chem. 6, 263-271

LUND, V., J. A. OLAFSEN (1998): Comparative study of pentraxin-like proteins in different fish species. Develop. Comp. Immunol. 22, 185-194.

MORIMATSU, M., A. WATANABE, K. YOSHIMATSU, T. FUJINAGA, M. OKOBO, M. NAIKI (1991): Elevation of bovine serum C-reactive protein and serum amyloid P component levels by lactation. J. Dairy Res. 58, 257-261.

MÜLLER, U., T. DALLE, G. LEUTHOLD, R. STAUFENBIEL, M. KRÜGER, C. KRETZSCHMAR, T. SEIDLER, M. F. G. SCHMIDT (1998): Züchtungsbiologische Charakterisierung der Beziechnungen zwischen Stoffwechselindikatoren und immunokompetenten Merkmalen bei hungerbelasteten Milchrindbullen. Arch. Tierz. Dummerstorf 41, 359-370.

NONNECKE, B. J., L. J. BURTON, M. E. KEHRLI (1997): Associations between function and composition of blood mononuclear leukocyte populations from Holstein bulls treated with daxamethasone. J. Dairy Sci. 80, 2403-2410.

NORDIN, G., I. SAMUELSSON, B. ANDERSSON, J. BORJESON (1996): C-reactive protein - The difference between quantitation in serum and EDTA plasma. Scand. J. Clin. Lab. Investig. 56, 123-127.

SHRIVE, A. K., G. M. T. CHEETHAM, D. HOLDEN, D. A. A. MYLES, W. G. TURNELL, J. E. VOLANAKIS, M. B. PEPYS, A. C. BLOOMER, T. J. GREENHOUGH (1996): Three dimensional structure of human C-reactive protein. Nature Structural Biol. 3, 346-354.

TOUSSAINT, M. J. M., A. M. VANEDEREN, E. GRUYS (1995): Implication of clinical pathology in assessment of animal health and in animal production and meat inspection. Comp. Haematol. Int. 5, 149-157.

WENG, X. D., G. CLOUTIER, R. BEAULIEU, G. O. ROEDERER (1996): Influence of acute phase proteins on erythocyte aggregation. Am. J. Physiol. 40, H2346-H2352.

WOLBINK, G. J., M. C. BROUWER, S. BUYSMANN, I. J. M. TENBERGE, C. E. HACK (1996): CRP-mediated activation of complement *in vivo* - assessment by measuring circulating complement-C-reactive protein complexes. J. Immunol. 157, 473-479.

ZHANG, D. X., M. SUN, D. SAMOLS, I. KUSHNER (1996): STAT3 participates in transcriptional activation of the C-reactive protein gene by interleukin-6. J. Biol. Chem. 271, 9503-9509.

ZIMMERMANN, S., A. NEUMANN, M. KRÜGER, M. FÜRL, K. ELZE (1998): Die Eignung einer klinischen Puerperalkontrolle sowie von Stoffwechsleparametern, unter besonderer Berücksichtigung des C-reaktiven Proteins, als Kriterium für das Konzeptsionsgeschen in folgenden Reproduktionszyklus des Rindes. Züchtungskunde 70, 261-281.

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SAŽETAK

C reaktivni protein (CRP) spada među važnije komponente akutne upalne reakcije u domaćih sisavaca. U ovom radu smo istraživali prisutnost CRP u slovenskih ovaca radi boljeg poznavanja njegove raspodjele. Kao referentni uzorak su poslužile 52 ovce (14 solčavske i 38 bovške pasmine) klinički zdrave, u dobi između šest mjeseci i četiri godine. Vrijednost CRP smo utvrđivali u krvnom serumu pomoću turbidimetrijskog imuno testa (TIA) s poliklonskim protutijelima kunića za humani CRP. Sve vrijednosti CRP su bile između 0,000 i 13,600 mg/l dok je prosječna koncentracija bila 6,280±0,429 mg/l. Referentne vrijednosti CRP (na razini 95% značajnosti) za uzorak smo ocijenili između 0,086 i 12,474 mg/l. Prosječne vrijednosti CRP za pojedine pasmine su bile 5,235±0,429 za bovšku i 9,115±0,647 mg/l za solčavsku ovcu ($P<0,001$). Ocjenjujemo da su vrijednosti CRP rezultat zajedničkog djelovanja metaboličkog i imunosnog sustava. Ovo istraživanje je vrijedno kao pomoć u razlikovanju fizioloških i patoloških vrijednosti CRP u ovaca.

Ključne riječi: turbidimetrijski imuno test, C reaktivni protein, referentne vrijednosti, ovca