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Decreased Apolipoprotein C-III Concentration in the High-Density Lipoprotein Fraction from Calves Inoculated with *Pasteurella haemolytica* and Bovine Herpes Virus-1

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ABSTRACT. Lipoprotein lipid and apoprotein concentrations are known to be altered during the acute-phase response. We have previously shown that the serum activity of lecithin:cholesterol acyltransferase (LCAT) and concentration of cholesteryl esters, both constituents of the high-density lipoprotein (HDL) fraction, are reduced in calves inoculated with *Pasteurella haemolytica* and bovine herpes virus-1, the two major pathogens for calf pneumonia. The concentration of apolipoprotein C-III (apoC-III), a low molecular mass protein component distributed mainly in the HDL fraction, was therefore examined in bacteria- and virus-inoculated calves. An enzyme-linked immunosorbent assay demonstrated that it was decreased by inoculations of *Pasteurella haemolytica* and bovine herpes virus-1. The decrease was detected as early as 1 day after inoculation in both groups. A decreased serum apoC-III concentration was also observed by immunoblot analysis. It was detected in the HDL fractions from the bacteria- and virus-inoculated calves, and HDL apoC-III concentrations in the inoculated calves were decreased compared with controls. These results, coupled with the previous findings on LCAT activity and the cholesteryl ester concentration, indicate that a decreased HDL concentration is one of the early events occurring during the acute-phase response evoked by infections with *Pasteurella haemolytica* and bovine herpes virus-1.—KEY WORDS: apolipoprotein C-III, bovine, high-density lipoprotein, pneumonia.

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Apolipoprotein C-III (apoC-III) is the low molecular mass protein component of lipoproteins and is distributed in human plasma in chylomicrons (CM), very low-density lipoprotein (VLDL) and high-density lipoprotein (HDL) fractions [11]. It is suggested to be involved in the regulation of the triglyceride metabolism [5] in the CM and VLDL and the activation of lecithin:cholesterol acyltransferase (LCAT), the enzyme responsible for esterification of cholesterol in HDL [6]. The major site for apoC-III synthesis is the liver and the synthesis is regulated by cytokines [4, 13]. In contrast to apoC-III in humans and laboratory animals, its counterpart in bovines shows a unique property with respect to the distribution in lipoprotein fractions; cow apoC-III is detected mainly in HDL, not in the CM and VLDL fractions [18]. The predominant distribution in the HDL fraction implies the greater relevance of cow apoC-III in HDL functions such as the activation of LCAT rather than in the triglyceride metabolism. Moreover, the cow apoC-III concentration is higher during midlactation than during the nonlactating stage, suggesting a function related to lactation [19].

Recent accumulated evidence indicates that lipoprotein concentrations are altered during the acute-phase response [1, 2]. The most typical changes in response to infection are increased VLDL triglyceride and decreased HDL cholesterol concentrations [1]. In calves experimentally inoculated with *Pasteurella haemolytica* and bovine herpes virus-1 (BHV-1), the two major pathogens for calf pneumonia [3, 12, 14, 16, 20], we have shown that LCAT activity and the cholesteryl ester concentration are decreased [10]. Because apoC-III also is distributed in the HDL fraction, it is conceivable that the apoC-III concentration is decreased in experimental pneumonia. The purpose of the study reported here was to evaluate the apoC-III concentration in calves inoculated with *Pasteurella haemolytica* and BHV-1, in order to elucidate the involvement of apoC-III in the pathogenesis of calf pneumonia, one of the most economically important disorders in calves.

MATERIALS AND METHODS

Induction of pneumonia: Pneumonia was experimentally induced by inoculations of *Pasteurella haemolytica* and BHV-1 into calf lung lobes, as described previously [7, 8, 10, 17]. Briefly, a suspension of *Pasteurella haemolytica* (serotype 1, I29 strain; 1×10^9 colony forming units) was administered to right lung lobes of 10 Holstein male calves (2 to 3 months old) using a fiberoptic bronchoscope. Ten other calves receiving the vehicle (phosphate-buffered saline [PBS]) alone were used as controls. Under xylazine- and procaine-induced anesthesia, calves were exsanguinated at day 1 (3 control and 3 inoculated calves), day 2 (3 control and 3 inoculated), day 4 (2 control and 2 inoculated) and day 7 (2 control and 2 inoculated). Blood was collected at 0 (1 hr before administration) and 0.25 (6 hr after) and at 1,

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2, 3, 4, and 7 days after treatment. BHV-1 (Los Angeles strain; 1×10^7 $TCID_{50}$) was administered to right lung lobes of 14 male Holstein calves (1.5 to 2 months old), as in the bacterial inoculation. Three other calves were used as controls for the virus-inoculated group. The 17 calves were exsanguinated on and after day 4. Blood was obtained at 0, 0.25, 1, 2, 3 and 4 days after treatment. Clinical symptoms observed after the bacterial and viral inoculations were as described previously [7, 8, 10, 17].

Purification and antibody production of apoC-III: ApoC-III was purified from cow serum [18], and its antiserum was raised in rabbits [19]. The antiserum produced was monospecific to apoC-III, as indicated by immunoblot analysis. The serum apoC-III concentration was determined by an enzyme-linked immunosorbent assay (ELISA) [19].

Other methods: CM (d<0.95), VLDL (d<1.006), low-density lipoprotein (LDL, d<1.063), HDL (d<1.21) and the d>1.21 fraction (containing very high-density lipoprotein and lipoprotein-deficient fractions) were prepared from calf sera as described previously [8, 15]. Protein concentration was determined by the method of Lowry *et al*. [9]. Significance was analyzed using a paired *t*-test. Values are expressed as mean ± SD.

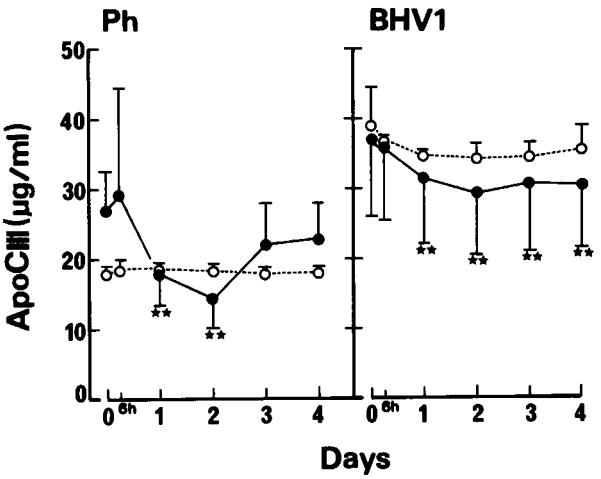

Fig. 1. Changes in the serum apoC-III concentration in calves experimentally inoculated with *Pasteurella haemolytica* (Ph) and BHV-1. Open circles, control; closed circles, inoculated. Sample sizes are: n=10 for day 0, 0.25 and 1, n=7 for day 2, and n=4 for days 3 and 4 in both control and Ph-inoculated calves; n=3 for the control and n=14 for BHV-1 inoculated calves. Because of the small sample size (n=2), values at day 7 in Ph-inoculated calves are not included. ***P*<0.01, compared with respective values for day 0.

RESULTS

The serum concentration of apoC-III was significantly decreased at 1 and 2 days after inoculation with *Pasteurella haemolytica* (Fig. 1). The concentration at day 2 was approximately half that at day 0, and thereafter returned to near the level before inoculation. The apoC-III concentration in the control group was not significantly changed during the experiment. The apoC-III concentration at day 0 in calves used for viral inoculation showed considerable individual variation, and was higher (although not significant) than that in calves used for the bacterial inoculation. The apoC-III concentration was also decreased in calves inoculated with BHV-1. The apoC-III concentration in the virus-inoculated calves remained decreased through 4 days after inoculation, although decreased rates were not as distinct as in the bacterial inoculation (at day 2, 78.5% that of day 0). No significant change of the apoC-III concentration in the control group was seen in the experiment using viral inoculation.

The decrease in the apoC-III concentration in calves inoculated with *Pasteurella haemolytica* was further confirmed by immunoblot analysis (Fig. 2). No apparent change in the intensity of the apoC-III band was observed in serum from a control calf. By comparison, intensities of apoC-III bands in sera from the bacteria-inoculated calves were decreased, particularly at days 1 and 2. Decreases in intensities of apoC-III bands were similarly observed in sera from calves inoculated with BHV-1 (figure not shown).

Before the bacterial inoculation, almost all apoC-III was found in the HDL fraction, and it was not or only faintly detected in the other lipoprotein fractions (Fig. 3). The distribution site of apoC-III was not affected by the bacterial inoculation, and most apoC-III was found in HDL. The intensity of the apoC-III band in the HDL fraction, as well as that in serum, was decreased at 1 day after inoculation. The analysis of lipoprotein fractions from calves inoculated with BHV-1 gave similar results (not shown).

DISCUSSION

The present results indicated that the serum apoC-III concentration was decreased by inoculations with *Pasteurella haemolytica* and BHV-1. The decreases were significantly detected as early as 1 day after inoculation in both cases, suggesting that a decrease in the apoC-III concentration is one of the early events during the acute-phase response evoked by infection by the two pathogens.

We have previously suggested that the apoC-III concentration is higher in Holstein female calves (2 to 4 weeks old) than in heifers (7 to 10 months old) [18]. The higher concentrations (Fig. 1) at day 0 in calves inoculated with BHV-1 (1.5 to 2 months old) than in calves inoculated with *Pasteurella haemolytica* (2 to 3 months old) may be explained, at least in part, by the age-related change of the apoC-III concentration. The decrease in the apoC-III concentration in calves inoculated with BHV-1 was not as distinct as in those inoculated with *Pasteurella haemolytica*. In a previous study [17], 1.5-month-old calves were inoculated with *Pasteurella haemolytica*. The apoC-III concentrations in calves at 1.5 months old of age were decreased as in the present study (the value at day 2 was nearly half of the value at day 0; Yamamoto and Katoh, unpublished results). The previous results indicated it is

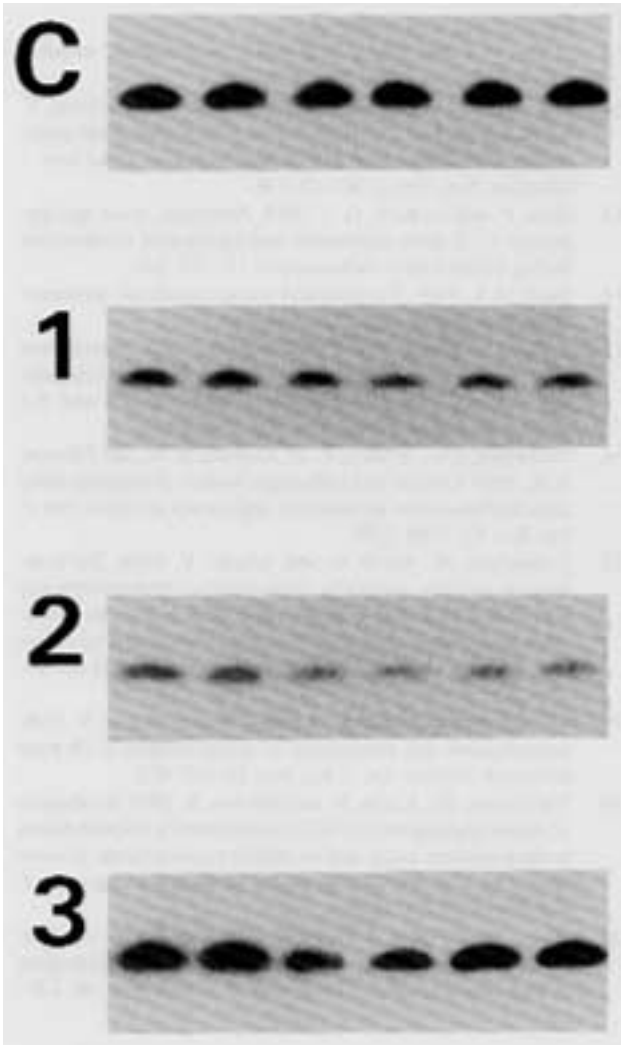

Fig. 2. Immunoblot analysis of apoC-III in sera of a control calf (C) and 3 calves inoculated with *Pasteurella haemolytica* (1–3). Lanes (left to right) are: 0 days, 0.25 days, 1 day, 2 days, 3 days and 4 days after treatment. A 6.67-μl aliquot of serum diluted 20-fold with PBS was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis using a Tricine-buffer system [18]. Blotted membranes were reacted with 5,000-fold-diluted anti-cow apoC-III rabbit serum and thereafter with 1,000-fold-diluted anti-rabbit IgG goat serum conjugated with horseradish peroxidase.

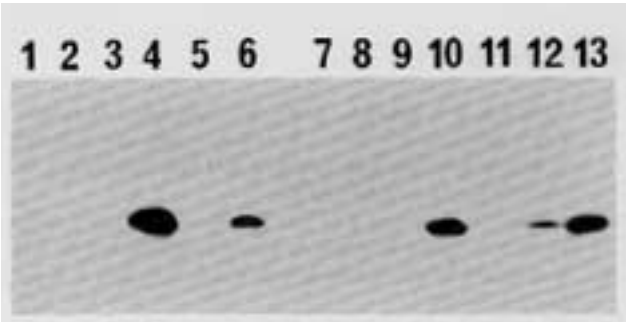

Fig. 3. Immunoblot analysis of lipoprotein fractions from sera of a control calf (1–6) and a calf inoculated with *Pasteurella haemolytica* (7–12). Lanes are: 1, 7, CM; 2, 8, VLDL; 3, 9, LDL; 4, 10, HDL; 5, 11, the d>1.21 fraction; 6, 12, serum; and 13, purified apoC-III (0.586 μg). Two-μl aliquots of 3-fold-diluted CM, VLDL, LDL and HDL, and 6.67-μl aliquots of 20-fold-diluted d>1.21 fraction and serum were applied. Other conditions were as described in Fig. 2.

unlikely that the apoC-III concentration in calves at 1.5- to 2-months of age is less sensitively decreased than that in calves at 2- to 3-months of age. The present results, coupled with the previous findings, suggest that BHV-1 is less potent than *Pasteurella haemolytica* in decreasing the apoC-III concentration under the conditions used. The hepatic synthesis of apoC-III is regulated by cytokines [4, 13]. Cytokines produced in response to infection may suppress the synthesis of apoC-III by the liver. The difference in the decreased rates of apoC-III concentrations in calves inoculated with *Pasteurella haemolytica* and BHV-1 may be explained by the different species and amounts of cytokines induced by infections by the two pathogens.

During the acute-phase response, HDL cholesterol concentration is decreased [1, 2]. The rationale for the decrease is thought to be suppression of the reverse-transport of cholesterol to the liver from injured tissues, in which cholesterol is required for repair and regeneration of damaged membranes. The concentration of cholesteryl esters, the product of the LCAT reaction, is decreased in HDL fractions prepared from sera of bacteria- and virus-inoculated calves [10]. ApoC-III acts as an activator of LCAT [6]. The decrease in the apoC-III concentration may contribute to suppress cholesterol esterification in HDL, and in turn lower cholesterol uptake from the lung.

In nonruminant animals such as humans, apoC-III is distributed in the CM and VLDL fractions as well as in HDL [11]. By contrast, cattle apoC-III is mainly detected in HDL, and is not or only faintly found in CM and VLDL [18], presumably because of a lower triglyceride concentration (approximately 1/10 of humans). The predominant distribution of apoC-III in HDL was not affected by the bacterial and viral inoculations. The conserved distribution, even in pathologic state, supports the hypothesis that cattle apoC-III is more relevant in HDL-oriented functions such as the activation of LCAT, than the triglyceride metabolism in CM and VLDL.

In conclusion, apoC-III in the HDL fraction was decreased in calves with pneumonia induced by *Pasteurella haemolytica* and BHV-1. The decreased apoC-III concentration, together with the reductions in LCAT activity and the cholesteryl ester concentration, suggests that the suppression of HDL concentration is involved in the pathogenesis of calf pneumonia.

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