Detection of Serum Total Sialic Acid in Cattle with Natural Tropical Theileriosis

T. ILHAN KARAGENÇ, F. KARGIN KIRAL, K. SEYREK, A. BILDIK and H. EREN

1 Department of Parasitology, Faculty of Veterinary Medicine, University of Adnan Menderes, Aydin, TURKEY
2 Department of Biochemistry, Faculty of Veterinary Medicine, University of Adnan Menderes, Aydin, TURKEY

Corresponding author : E-mail : kmseyrek@hotmail.com

SUMMARY

The aim of this study was to determine the serum concentrations of total sialic acid in cattle naturally infected with Theileria annulata before and after treatment. Sixteen diseased cattle and thirteen control animals were included in the present study. Theileria infection was confirmed both with Giemsa’s staining of blood smears and by showing the presence of Theileria annulata using reverse line blot (RLB) hybridisation with polymerase chain reaction (PCR) amplified region of 18S rRNA gene. Compared to the control animals (1141.38 ± 68.14 µg/ml), a marked increase of serum total sialic acid concentrations (2520.90 ± 147.94 µg/ml) was obtained in infected cattle (p < 0.001). Three months after treatment, total serum sialic acid concentrations significantly decreased (1502.71 ± 116.13 µg/ml, p < 0.001). However, they remained significantly higher than in controls (p < 0.05). It can be concluded that Theileria annulata infection induced marked and persistent elevations of serum sialic acid concentrations, suggesting that sialic acid would indirectly promote the setting and remanence of the parasite in the host.

Keywords : Cattle - theileriosis - sialic acid.

Introduction

Sialic acids are nine-carbon monosaccharides that link to the terminal galactose, N-acetylgalactosamine, or to other sialic acids in carbohydrate chains of glycoproteins or glycolipids [5]. Sialic acids usually occupy exposed terminal positions on the oligosaccharide chains of glycoconjugates and frequently serve as ligands for receptors such as selectins and siglecs, which mediate a variety of cell-cell adhesion processes in the inflammation and in the immune response [17, 19].

Tropical theileriosis is an important and frequently fatal disease of cattle caused by the protozoon parasite Theileria annulata [24]. Tropical theileriosis particularly threatens exotic Bos taurus breeds of European origin and it may cause 40-60% mortality [1]. When cattle survive to infection, recovery is extended and is often incomplete, resulting in loss of productivity and a carrier state [30]. Therefore, bovine tropical theileriosis imposes serious constraints upon livestock production.

Sialic acid is present in normal human and cattle serum. It has been demonstrated that sialic acid concentrations are elevated in patients suffering from various diseases [22, 26, 32]. However, the effect of Theileria infection on serum sialic acid content in cattle has not been reported previously. Therefore, the aim of the present study was to examine if there is any relationship between theileriosis and the serum total sialic acid concentrations and to verify if serum sialic acid concentrations correlate with the severity of the infection.

Material and Methods

1-ANIMALS AND SAMPLES

Twenty-nine Holstein-Fressein cattle, (24 females and 5 males), 3 month to 8 year old, grazed in the pastures in Aydin region were used in this study. Sixteen cows were infected naturally with T. annulata and thirteen control animals were clinically healthy. All the cattle included in this study were submitted to clinical and parasitological examinations. All of the diseased animals showed some or all clinical signs of acute theileriosis including fever, enlargement of lymph nodes, inappetence, drooling from mouth, serous nasal discharge, swelling of the eyelids, and drop in milk production.

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Treatment of sixteen infected animals was conducted with buparvaquone (Butalex®, Schering-Plough/England) once at a dose of 2.5 mg/kg, intramuscularly. Blood samples were taken into plain and EDTA containing vacutainer tubes from the jugular vein. Samples were firstly taken at the onset of the disease during the disease season of theileriosis (June-July), then 3 months following the treatment of diseased animals. At this time, the treated animals did not show any clinical signs of theileriosis. Because serum samples could not be obtained from 4 out of 16 diseased animals following treatment, the serum sialic acid concentrations were only measured on 12 animals. Serum samples were obtained after centrifugation at 1700 g for 15 minutes at room temperature, aliquoted and stored at -20°C until used.

2. CLINICAL AND PARASITOLOGICAL ANALYSIS

Serum total sialic acid concentrations were measured as described by SYDOW et al. [29]. Briefly, 400 µl of serum were treated with 3 ml of 5% perchloric acid for 5 min at 100°C and centrifuged at 1400 g for 4 min. The supernatant (2 ml) was mixed with 400 µl of Ehrlich reagent (5 g p-dimethylaminobenzaldehyde / 50ml HCl / 50ml distilled water). After incubation at 100°C for 15 min, 2 ml of distilled water were added on sample and a spectrophotometer (Shimadzu, UV-1601) was used to read the optical density at 525 nm. A standard curve was obtained using known quantities of fresh N-acetylneuraminic acid (Sigma, A-0812) dissolved in water.

EDTA-blood was used to prepare thin blood smears and to extract DNA for the reverse line blot (RLB) hybridisation analysis. Blood smears were fixed in methanol, stained with Giemsa and examined for the presence of blood protozoa. The degree of parasitaemia was recorded as the percentage of the infected red blood cells after counting 1000 cells. The smear was recorded as negative if no piroplasms were observed in 200 fields.

DNA from either infected or uninfected blood samples was extracted as described previously [9]. Briefly, 200 µl of blood were washed three times with 0.5 ml of lysis buffer (37.6 mM NaCl, 1 mM EDTA, 0.015% saponin) by centrifugation at 14,000 g for 5 min, resuspended in 100 µl of PCR mixture (10 mM Tris-HCl pH 8.0, 50 mM KCl, 0.5% Tween 20, 100 (g proteinase K / ml), and incubated overnight at 56°C. Samples were then heated for 10 min at 100°C and centrifuged at 14,000 g for 2 minutes.

The amplification of 18S rRNA gene of Theileria and Babesia by PCR was conducted as described previously [13]. The forward primer, RLB-F (5’- GACACAGGGGAGG-TAGTGACAAG) and the reverse primer RLB-R (biotin-5’-CTAAGAATTTCACCTGACAGT) amplified regions in both Theileria and Babesia genus were used. Specific oligonucleotides, which contained an N-terminal C6 Amine linker, were used for Theileria and Babesia genus (5’- TAATGGTTAATAG-GARCRGTGT : T. annulata / 5’- CTTCCGGGTTCTGTGCA : T. buffeli / 5’- GGCTTATT-TCGGWTTGATTTT and 5’- CAGGTTTCGCCTGTA- TAATTGAG : B. bovis) and were covalently linked to the Biodyne C support membrane and hybridisation was carried as described elsewhere [13, 15].

3. STATISTICAL ANALYSIS

Sialic acid differences among groups were analysed by one-way ANOVA followed by Duncan test for multiple comparisons. Pearson correlation coefficient (SPSS version 12.0), was used to analyse the correlation between the degree of parasitemia and serum sialic acid concentrations. A value of p<0.05 was considered significantly different.

Results

Blood smears prepared from the 16 diseased animals showed the presence of piroplasm of T. annulata in the red blood cells with different parasitaemia (between 2% and 35%). On the other hand no piroplasm was detected in control animals. After recovery (i.e. following the treatment), piroplasms were still detected in all infected animals by direct microscopic examination with very low degree of parasitemia (between 0.1% and 0.5%). The RLB hybridisation demonstrated that control animals were free of T. annulata, T. buffeli and B. bovis, whereas it confirmed the presence of Theileria annulata in all infected animals before and after treatment. However, the intensity of signal in RLB was slightly reduced in treated animals (Figure 1).

Figure 1. — RLB results for blood samples. Probes : A- Theileria and Babesia species ; B- Theileria annulata ; C- Theileria buffeli ; D- Babesia bovis. Samples : 1. Negative control DNA ; 2. Babesia bovis control DNA ; 3. Theileria buffeli control DNA ; 4. Theileria annulata control DNA ; 5.7-18,20-22 : cattle infected with Theileria annulata ; 6 and 19 : animals free of Theileria and other protozoa.

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Mean serum sialic acid concentrations were significantly higher in the diseased animals than in the control and treated animals \((p < 0.001)\). A significant decline of serum sialic acid concentrations was evidenced in the treated animals (from 2520.90 ± 147.94 µg/ml to 1502.71 ± 116.13 µg/ml). Despite this decline, sialic acid concentrations were still significantly increased in sera of treated animals (3 months after treatment) in comparison to control animals \((p < 0.05, \text{Table I})\). However, the individual rates of parasitemia were not correlated with sialic acid concentrations \((r = -0.027, p > 0.05)\).

### Discussion

The *T. annulata* infection starts with the introduction of sporozoites delivered by an infected tick during blood meal into the host blood stream. Sporozoites rapidly enter mononuclear cells or other target cells displaying major histocompatibility complex (MHC) class II molecules [2, 14, 28]. Then, the parasite differentiates into a multinucleate macroschizont, leading to immortalisation and transformation of the infected cell [20]. Some macroschizonts subsequently differentiate to merozoites. Once released from the host cell, merozoites enter erythrocytes to form piroplasms [20]. Animals exposed to the parasite either die or become immune [1].

This study was conducted to examine the effect of *Theileria* infection on total serum sialic acid concentrations. *Theileria* infection has induced a significant increase of total serum sialic acid concentrations. Furthermore, treatment of animals significantly reduced serum sialic acid concentrations. Nevertheless, despite the significant decline in serum sialic acid content following the treatment, sialic acid concentrations remained significantly elevated up to three months following the treatment. Since it is well known that after recovery, animals become carrier of the parasite [16, 30], such high concentrations of sialic acid were probably due to the carrier state of treated animals.

It is currently unknown how infection with *Theileria annulata* leads to increases of serum sialic acid content. However, infections with various parasites such as *Leishmania* [3], and *Trypanosoma* [10] are also associated with elevated serum sialic acid concentrations. Sialic acid could modulate biological cell-cell interactions in two non-mutually exclusive ways. First, sialic acid could mask the underlying sugar chains (i.e. lactosaminic sequences), hindering then from interacting with galactose-specific lectins (galectins) [25]. Second, sialic acid would directly interact...

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### Table I

<table>
<thead>
<tr>
<th>Control group ((n = 13))</th>
<th>Infected group ((n = 16))</th>
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<tr>
<td></td>
<td>During disease ((n = 16))</td>
<td></td>
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<tr>
<td></td>
<td>After treatment ((n = 12))</td>
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<tr>
<td>Sialic Acid ((\mu g/ml))</td>
<td>1141.32 ± 68.14*</td>
<td>2520.90 ± 147.94b</td>
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*a, b, c*: Different letters indicate statistically significant differences in the same line.

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**FIGURE 2.** — A diagram showing the potential roles of sialic acid during the *T. annulata* infection. SA: Sialic Acid, +: positive action, -: negative action.
with specific sialic acid-binding lectins (siglecs) [6, 18, 21]. Therefore, increased contents of sialic acid would interfere with the attachment of sporozoites on host cells, or promote the invasion of erythrocytes by merozoites.

An important feature of tropical theileriosis is the establishment of foci of infected-cells with replicating macroschizonts in various organs due to the metastasis of macro-schizont-infected cells [11, 12]. In this context, as marked elevations in serum sialic acid concentrations occur in several types of malignancy [12, 32], and because increased sialylation of cell surface glycoproteins is shown to promote the metastatic potential and invasiveness of cancer cells [12, 32], sialic acid would also enhance metastatic potential and invasiveness of macroschizont-infected cells, during the establishment and the maintenance of *T. annulata* infection in cattle.

Infection with *T. annulata* stimulates various immune responses [26] and particularly natural killer (NK) cells would play an important role in the innate immunity to *T. annulata* infection. Indeed, NK cells lyse schizont-infected cells and produce IFN-γ which activates uninfected macrophages to produce TNF-α and NO [26]. But the cell surface hypersialylation hides some antigens, decreasing infected cell susceptibility to NK cells, and reducing the host immune response [4, 23, 27]. Therefore, the elevations of serum sialic acid concentrations observed in *T. annulata* infected cattle may compromise the host immune response to various stages of the parasite and promote cell invasion by *T. annulata* (Figure 2). Elevated sialic acid contents may as well alter receptor-ligand interactions between the sialic acid and its receptors such as selectins and siglecs, which are known to play important roles in the inflammation and in the immune response [4, 7, 8, 23, 27].

Taken together, this study demonstrates that natural infection of cattle with *T. annulata* leads to significant increases of total serum sialic acid concentrations and that this marker remains elevated even three months after the treatment of diseased animals. Further studies are required to analyse the type of sialilation (α-2,3 or α-2,6) and to identify the origin of the increase of the serum sialic acid content in blood.

References


