Alternative pathway of complement activation (APCA) in different sheep breeds

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Material and methods

ANIMALS:

- The sheep included in this study were from 3 productive types:
  A. Milk type crossings: 118 sheep and 14 rams - Stara Zagora - East-Friesian and (Stara Zagora - East-Friesian) - Blackhead Pleven breed;
  B. Merino type: 80 sheep and 9 rams from the Trakia merino breed;
  C. Meat type: 107 sheep from the Ile de France breed and 107 ewes and 6 rams from the Charollais breed;
  D. Furthermore, 45 lambs (21 male and 24 females - dairy crossings) were included in the study.

- For family analysis, rams and ewes (milk type) were selected according their APCA activities. They presented high and middle APCA activities, and we could not find out animals with low APCA activity (i.e. below 80 CH50). After mating of breeders, APCA activities were determined on each progeny.

- The cited facts evidence the considerable significance of APCA for systemic defense against various pathogens. The objective of the present study was to determine the occurrence of APCA differences among various sheep breeds, in adults and in lambs, before employing this parameter as a marker of immune defenses during infectious diseases.

SUMMARY

Sera from 486 sheep belonging to four breeds (Milk type crosses, Trakia merino, Charollais, Ile de France, and lambs from dairy crossings) have been tested for determining intensity of alternative pathway of complement activation (APCA). The highest average values of APCA were obtained in Ile de France sheep and Milk type rams. Breed related differences for this parameter was evidenced but variations dependent on the gender were contradictory. Besides, APCA concentrations in lambs were partially influenced by APCA status of parents, suggesting that selection of breeders based on this parameter would improve innate immune resistance in progeny.

Keywords: natural immunity - complement - alternative pathway - sheep - breeds - lambs.

RÉSUMÉ

Intensité de l’activation du complément par la voie alterne dans différentes races ovines. Par L. SOTIROV, I. DIMITROV, M. DJORBINEVA et S. TANCHEV.

Le degré d’activation du complément par la voie alterne a été mesuré dans 486 sérum de moutons appartenant à 4 races (croisements lattiers, Mérinos de Thrace, Charollais, Ile de France et chez des agneaux lattiers). Les valeurs moyennes maximales ont été observées chez les brebis Ile de France et chez les béliers de type laitier. Des différences dues à la race ont été mises en évidence, mais l’influence du sexe sur le degré d’activation du complément n’a pas été univoque. De surcroît, l’intensité de l’activation du complément par la voie alterne chez les agneaux est partiellement reliée aux statuts parentaux ce qui suggère qu’une sélection des reproducteurs sur ce paramètre pourrait améliorer la résistance immune innée des nouveau-nés.

were 2-3-year-old. They were housed in separate premises. Blood for analysis was sampled in 10 mL tubes from \textit{v. jugularis}. The blood was allowed to clot for one hour at room temperature (25°C) and the samples were centrifuged at 2000 g for 10 min.

**APCA determination:**

The alternative pathway of complement activation (APCA) was studied by the method of SOTIROV [16]. Each serum sample was first diluted by mixing 100 mL serum with 350 mL veronal - veronal Na buffer (in final concentrations: 146 mM NaCl, 1.8 mM 5,5-diethylbarbituric acid sodium salt, 3.2 mM 5,5-diethylbarbituric acid, 1 mM EGTA and 0.8 mM MgCl2). In U bottomed plates (Flow Laboratories, UK), 7 other dilutions from each diluted serum were again prepared in veronal-veronal Na buffer: 80 mL diluted serum + 20 mL buffer, 70 mL diluted serum + 30 mL buffer, 60 mL diluted serum + 40 mL buffer, 50 mL diluted serum + 50 mL buffer, 40 mL diluted serum + 60 mL buffer, 30 mL diluted serum + 70 mL buffer and 20 mL diluted serum + 80 mL buffer. The final serum dilutions were respectively 8/45, 7/45, 6/45, 5/45, 4/45, 3/45 and 2/45. Then, 50 mL buffer and 100 mL of 1% rabbit erythrocyte suspension were added to each well. After incubation for 1 hour at 37°C, samples were centrifuged at 150 g for 3 minutes at room temperature (23°C). Thereafter, 150 mL of each supernatant were removed and placed in flat bottomed plates for measurement of optic density at 540 nm by “Sumal-PE2” ELISA reader (Karl Zeiss, Germany). The final APCA activity was calculated using special computer programmes obtained after the crossing over of the IIIC/j and IIIVO/J lines, probably due to the heterosis effect.

**STATISTICAL ANALYSIS:**

Data were analysed using the fixed effect MANOVA model (Program Statistica, Statsoft, Inc., USA) and evaluated through the following formula:

\[
y_{ij} = m + a_i + e_{ij}
\]

where \(y_{ij}\) is the observation value of the investigated trait, \(m\) the population mean, \(a_i\) the breed effect and \(e_{ij}\) the random errors. Differences were considered as significant when \(p\) values were less than 0.05.

**Results and discussion**

Four sheep breeds from different productivity type were used in the present study. The results are presented in Table I. The Ile de France sheep presented the highest APCA activity, whereas the lowest activity was observed for Charollais breed, and particularly for rams (the difference between Ile de France and Charollais breeds was statistically significant : \(p < 0.001\)). The rams from dairy type presented similar complement activity as the Ile de France sheep and the differences with Charollais rams and ewes were also significant (\(p < 0.05\) and \(p < 0.01\), respectively). Intermediate activities were found for Merino breed and milk type sheep.

Similar results were communicated by AUDRAN et al. [2]. They studied 5 sheep breeds : Préalpe, Ile de France, Limousine, Mérinos d’Arles and Bouchara and reported the highest complement activities in Ile de France sheep (171.4 CH50) followed by Préalpe (168.8 CH50) and Mérinos d’Arles sheep (167.7 CH50), but the differences among these breeds were not significant. The average complement activities in Limousine (159.6 CH50) and Bouchara (138.1 CH50) sheep were however statistically lower. The breed-related differences showed that the APCA activities were under a genetic control. Dissimilarities related to the breed were observed in other animal species too. FOX et al. [7] have studied the cytotoxic effect of rabbit complement on sensibilised murine lymphocytes. The source of complement was stemmed from various rabbit lines. It was shown that out of the pure lines, the highest lymphocyte activity was that of rabbits from the IIIC/J and IIIVO/J lines whereas the lowest (from the IIIVO/vptj line. The highest cytotoxic effect was observed after the crossing over of the IIIC/j and IIIVO/J lines, probably due to the heterosis effect.

**TEDESCO and LACHMAN** [17] reported that the normal levels of C6 in rabbits were 35 g/L. ABE et al. [1] were the first to detect a complete C6 deficiency in Angora rabbits, i.e. to prove that they were homozygous by respect to the C6° gene.

### Table I

<table>
<thead>
<tr>
<th>Breeds</th>
<th>n</th>
<th>Mean ± SE</th>
<th>VC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk type sheep</td>
<td>118</td>
<td>133.68 ± 2.04^bc</td>
<td>16.50</td>
</tr>
<tr>
<td>Milk type rams</td>
<td>14</td>
<td>149.71 ± 6.63^bc</td>
<td>16.68</td>
</tr>
<tr>
<td>Trakia Merino sheep</td>
<td>80</td>
<td>147.98 ± 2.73^abc</td>
<td>16.42</td>
</tr>
<tr>
<td>Trakia Merino rams</td>
<td>9</td>
<td>131.25 ± 5.62^bc</td>
<td>12.11</td>
</tr>
<tr>
<td>Ile de France sheep</td>
<td>107</td>
<td>153.05 ± 2.00^a</td>
<td>13.48</td>
</tr>
<tr>
<td>Charollais sheep</td>
<td>107</td>
<td>130.57 ± 1.98^ab</td>
<td>15.62</td>
</tr>
<tr>
<td>Charollais rams</td>
<td>6</td>
<td>125.4 ± 8.11^a</td>
<td>14.47</td>
</tr>
<tr>
<td>Male lambs</td>
<td>21</td>
<td>125.04 ± 4.44^a</td>
<td>10.66</td>
</tr>
<tr>
<td>Female lambs</td>
<td>24</td>
<td>123.04 ± 2.79^a</td>
<td>9.64</td>
</tr>
</tbody>
</table>

*Table I.* — APCA activities in different sheep breeds (expressed as CH50). Results are expressed as means ± standard error (SE). VC(%) : Coefficients of variations. ^abc Mean values with different superscripts within a column differ significantly (\(p < 0.05\)).

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Table II. — Average APCA activities (expressed as CH50) in milk type rams, dams and their progenies

<table>
<thead>
<tr>
<th>Rams</th>
<th>APCA</th>
<th>number</th>
<th>Dams</th>
<th>APCA</th>
<th>number</th>
<th>Lambs</th>
<th>APCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>98</td>
<td>120.23</td>
<td>5</td>
<td>120.28 ± 10.85</td>
<td>8</td>
<td>142.67 ± 7.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0409</td>
<td>123.71</td>
<td>5</td>
<td>162.28 ± 20.16</td>
<td>8</td>
<td>117.51 ± 1.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9512</td>
<td>143.61</td>
<td>5</td>
<td>122.27 ± 9.83</td>
<td>7</td>
<td>118.71 ± 3.34&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0410</td>
<td>181.05</td>
<td>5</td>
<td>155.70 ± 40.11</td>
<td>8</td>
<td>123.67 ± 12.58&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>181.05</td>
<td>12</td>
<td>127.25 ± 4.96</td>
<td>14</td>
<td>124.76 ± 3.76&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HINZOVA et al. [10] thought that the genes controlling the levels of complement in mice, were related to the H-2 complex. This suggestion was further confirmed by DEMANT et al. [6] and CAPKOVÁ and DEMANT [4], who have proved that the genes determining the synthesis of the Ss-S1p protein were in the H-2K locus. Later it was pointed out that the Ss-S1p component was identical with the C4 component [13 - 15]. GOLDMAN and GOLDMAN [8] found out that the H-2 complex controlled the expression of the early components of complement.

WAMBURA et al. [18] compared 3 zebu breeds (Meru, Mbullu, and Iringa Red) and their crosses with Friesian cattle by respect to their resistance against ticks. It was observed that purebred zebu were more resistant to parasites compared to crossbreds. Also, the activity of complement in purebred zebu was higher than that in crossbreds. It was assumed that the highest complement activity was important for the higher resistance of the three zebu breeds. This suggestion was supported by the statistically significant negative correlation between the degree of infection with parasites and the complement activities.

Despite the observed breed-related differences, there are probably variations dependent on the gender, but with this regard, our results were contradictory. Trakia Merino and Charollais rams had a lower APCA activity than females, but differences were not significant. By contrast, rams from the dairy type showed a similar APCA activity as Ile de France sheep and the difference with Charollais sheep was significant (p < 0.05). But again, in this milk type, the difference between males and females was not significant. In both male and female dairy lambs, the activities were almost identical (males : 125.04 ± 4.44 CH50; females : 123.04 ± 2.79 CH50). All those facts did not support the hypothesis for gender-related differences in complement activities.

The aforementioned facts emphasized the importance of APCA for the defense of animals against pathogenic agents and the possibility for a serious genetic control on this important humoral factor. A family analysis was performed in order to elucidate the effects of parents upon the inheritance of the factor by the progeny. There was a definite phenotypical diversity of APCA in the various types of animals: in mothers - between 120.68-181.05 CH50; in fathers - between 120.68-181.05 CH50 and in offsprings - between 102.68-143.82 CH50. Generally, the complement system is a conservative and relatively stable parameter. When we studied how the APCA activity was inherited in the progeny, we noticed that offsprings tend to exhibit relatively high APCA values when their parents had already high values (Table II). For example, 14 lambs from ram “A” (which presented the highest complement activity) showed values equal or above the average value of the total progeny (124.76 ± 3.76 CH50). However, the highest APCA values (142.67 ± 7.15 CH50) were observed for ram 98’s progeny, although this male exhibited the lowest APCA concentration among rams (Table II). The differences between ram 98’s progeny and other lambs stemmed from other rams were significant (p < 0.05). Besides, no positive significant correlation between APCA concentrations of lambs and fathers was found. It was probable that some other factors, such as mother influence, would interfere in the determination of APCA activity in lambs. But again, even if contribution of dams would be out of importance in APCA status, it was not sufficient for explaining the great APCA variations encountered in lambs. Indeed, APCA activities in progenies did not significantly correlate with APCA activities of mothers, and APCA activities have greatly differed among lambs belonging to a same litter.

As conclusion, our results demonstrated that, firstly, the APCA in the studied sheep breeds were different, and secondly APCA activities in lambs would partially be related to APCA status of parents. Consequently, by selection of breeders, values of this parameter would be increased in progeny for achieving a higher innate immune resistance in lambs.

References