A Suspected Case of Neutrophil Dysfunction in a Holstein Heifer
Hajime NAGAHATA, Hiroshi NODA, Kiyoshi TAKAHASHI, Takashi KUROSAWA, and Mitsuo SONODA
Department of Animal Health, Department of Veterinary Internal Medicine, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069, Japan
(Received 22 May 1987/Accepted 29 July 1987)

KEY WORDS: Holstein heifer, neutrophil dysfunction.

Little is known about the occurrence of neutrophil dysfunction in animals. In 1983, Hagemoer et al. [5] reported one of the neutrophil dysfunction in cattle, bovine granulocytopenia, in a Holstein heifer. During a few years, we observed the three cases of bovine granulocytopenia in Holstein calves and reported previously [7]. The characteristic signs of bovine granulocytopenia were persistent or recurrent pneumonia, stunted growth, stomatitis associated with multiple ulcerative mucosae and chronic diarrhoea. Haematological examination revealed a marked, persistent neutrophilia (5–11×10^4/μl). The overall condition of the affected animal did not improve during the observation period and they all died. Details of the clinical findings of this syndrome have been described previously [11]. Neutrophils from affected animals with granulocytopenia showed a markedly decreased phagocytosis, bacterial killing activity, nitroblue tetrazolium (NBT) reducing activity, luminol-dependent chemiluminescence (CL) response and chemotactic response, as previously described [6, 7]. Recently, we observed a case of suspected neutrophil dysfunction in a fifteen-month-old Holstein heifer, which had clinical signs closely resembling those observed in bovine granulocytopenia. Animal was raised in a local dairy farm, Hokkaido, Japan. The heifer was fed a proprietary concentrate and hay. The clinical signs such as stunted growth, depression, pyrexia and stomatitis associated with ulcerative mucosae were first observed when the first diagnosing day, and heifer was treated with antibiotic therapy during one week period, however, the general condition did not improve and was referred to this department for the investigation. This communication describes the findings of neutrophil functions of affected heifer as compared with those of normal animals.

Blood was collected from jugular vein into evacuated tubes containing heparin (20 units/ml of blood). Polymorphonuclear cells (PMNs) were separated from the heparinised blood of affected and control animals by Ficoll-Conray density centrifugation [6]. The cell isolation procedure generally yielded a neutrophil preparation of more than 80–90 per cent purity. To determine the function of neutrophils, NBT reduction assay, luminol-dependent CL assay, chemotaxis assay and pololisation assay were performed. Quantitative NBT assay: Assay was performed according to the method described previously [6]. The assay system consisted of 200μl of 0.1 per cent NBT, 200μl of PMNs (1×10^6 cells) and 40μl of opsonised zymosan (10mg/ml) or Earle’s solution. After incubation for 15 min at 37°C, the reaction was stopped by adding 3ml of 0.5N HCl1 and washing twice with 0.5N HCl (1000, 5min) and then 3ml of dimethylformamide (DMF) were added. The formazan was extracted by boiling in water for 10min. Two ml of KOH (10N) were added and the optical density (OD) of formazan was read at 710 nm against DMF as blank. Delta (Δ) was calculated by subtracting the resting OD from the OD stimulated by opsonised zymosan. The results were expressing as ΔOD/1×10^6 PMNs/15 min in 3ml of DMF. Luminol dependent CL assay: Whole blood CL assay was performed according to the method of Tono-oaka et al. [12]. Each blood sample (100 μl) was added to 400 μl of Eagle’s medium in a measuring vial. After incubating the sample at 37°C for 20 min, it was placed in the measuring chamber of the chemiluminometer (Luminescence reader, Aloka BLR 102, Tokyo) and 10 μl of luminol (2mg/ml) was added. Following equilibration for 10 min, a background CL was measured for 5 min and then 10 μl of zymosan (50mg/ml) was added. A CL curve and an absolute peak response were read from the recorder. The CL index was calculated as follows: [peak CL (counts per minute: CPM)/number of PMNs in 100μl of blood]×1000. Chemotaxis assay: The chemotaxis assay under agarose was performed according to the method of Nelson et al. [9]. The chemotactic index (CI) was calculated as follows: linear distance moved from the margin of the well toward the outer well (chemotaxis)/linear distance moved from the margin of the well toward the inside (random migration). Polarisation assay: Assay was performed according to the procedure as described by Forsell et al. [4]. Briefly, 100 μl of PMNs (1×10^6cells) were mixed with 20μl of zymosan treated serum in culture tubes. After incubation at 37°C for 10 min, 2ml of 10 per cent formalin in phosphate buffered saline were added to the mixture and then incubated in ice-cold water for 30 min. The percentage of polarised cells was determined by observing 200 cells per sample under the microscope.

Total leucocyte counts in affected animal were in the range of 5.84 to 6.63×10^7/μl with 82 to 85 per cent neutrophils. Clinical laboratory findings revealed hyperglobulinemia with elevated gamma-globulin fraction (data not shown). Other haematological and clinic-pathological findings were approximately within
normal range. Results of neutrophil functions were shown in Table 1.

An important activity of phagocytes is their ability to respond to stimuli by activation of the respiratory burst [2]. One method to measure respiratory burst activity is by the reduction of NBT to formasan by the superoxide anion. The value of NBT reducing activity of neutrophils from affected heifer was remarkably decreased. Measurement of the oxygen-dependent events by CL analysis is now widely employed to reliably assess phagocytosis and overall O₂ redox metabolism in activated neutrophils, especially with PMN from humans [3, 10, 13]. During the process of phagocytosis and subsequent killing, phagocytic cells generate a burst of oxidative metabolic activity which results in an increased production of several bactericidal oxygen metabolites [1]. CL response of neutrophils from affected heifer appeared to be normal, but CL index [CL response per neutrophil] was extremely low as compared with those of controls. This indicated that phagocytic and/or bactericidal activity of neutrophils from affected heifer was impaired. In a previous study [8], we confirmed that the role of plasma in whole blood CL is apparently to opsonise zymosan with blood plasma, and the time it takes to attain the peak CL is determined mainly by the opsonic activity in blood. The time that took to show the peak CL was 3 times longer than that of controls in the present study, suggesting that the serum opsonic activity had decreased considerably. The polarised-pseudopod forming response to zymosan-activated serum was almost same to that of controls. This indicated that the ability to respond against chemotactic factors was satisfactory, although chemotactic movement, measured by under agarose, was decreased considerably.

These findings were consistent during the observation period and heifer was died at 48 days after admission. Necropsy revealed chronic pneumonia and extensive stomatitis associated with ulcer. These findings were similar to those of granulocytopenia in cattle as reported previously [5, 7, 11]. Form these findings suggested that the function of the neutrophils from affected heifer was impaired. Increased susceptibility to infection in the affected animal seems to be due to neutrophil dysfunction.

ACKNOWLEDGEMENTS. The authors are grateful to Drs. T. Tono-o and T. Matsumoto of the Pediatric Department, Otaru City Hospital, Hokkaido, for helpful discussions on the measurement of chemiluminescence response.

REFERENCES

10. Robinson, P., Wakefield, D., Breit, S. N., Eas-

<table>
<thead>
<tr>
<th>Date</th>
<th>WBC/μl</th>
<th>Neutrophils/μl</th>
<th>Peak CL (cpm)</th>
<th>CL index</th>
<th>T, S, CL (min)</th>
<th>NBT (ΔOD)</th>
<th>Random (mm)</th>
<th>Chemotaxis (nm)</th>
<th>C,F</th>
<th>Polarisating response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affected</td>
<td>17 June</td>
<td>66300</td>
<td>56620</td>
<td>31200</td>
<td>5.51</td>
<td>30.0</td>
<td>ND</td>
<td>1.12 ± 0.21 ± 0.26</td>
<td>1.04</td>
<td>ND</td>
</tr>
<tr>
<td>animal</td>
<td>11 July</td>
<td>58400</td>
<td>48180</td>
<td>4800</td>
<td>1.00</td>
<td>30.8</td>
<td>0.16</td>
<td>1.03 ± 0.22</td>
<td>1.03</td>
<td>94.0</td>
</tr>
<tr>
<td>Controls</td>
<td>(n=4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Leucocyte counts, chemiluminescence response, NBT-reducing activity, chemotactic response and polarising response of neutrophils from affected heifer and control animals

a) CL index = Peak CL (cpm) x 1000
b) T, S, CL = Time showing peak CL
c) C,F = Chemotaxis Random migration
d) Not determined
e) Mean ± SD
SUSPECTED CASE OF NEUTROPHIL DYSFUNCTION

1167


要約

好中球機能不全が疑われたホルスタイン若牛の1例（短報）：永澤・野田・高橋清志11・黒沢・隆11・其田三夫11（酪農学園大学家畜衛生学教室，11家畜内科学教室）—持続性肺炎、口底粘膜の潰瘍および高度の好中球増多症を伴うホルスタイン若牛（15か月齢）の好中球機能を検討したところ、著明なNBT還元能、ルミノール依存性化学発光反応および走化能の低下を認め、好中球の機能不全が疑われた。