Chemiluminescence Response of Cervine Neutrophils to Various Stimuli

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Abstract. The chemiluminescence response of cervine neutrophils to various stimuli was investigated in comparison with that of bovine and human homologues. The cervine cells showed a strong and consistent response, as observed in other species, to opsonized zymosan, phorbol myristate acetate and concanavalin A. The cells, however, like bovine homologues, failed to respond to n-formyl-methionyl-leucyl-phenylalanine which is a potent stimulant of human neutrophils. — Key words: chemiluminescence, neutrophil, Sika deer.


Neutrophils play an essential role in host defence as a primary cellular component against various infectious agents such as bacteria or fungi. The cells respond to the agents by enhancing cellular functions such as chemotaxis, phagocytosis and the oxidative burst [9]. While those functions are regarded as fundamental and universal, the response to stimuli is known to slightly differ among species. For example, human neutrophils respond to n-formyl-methionyl-leucyl-phenylalanine (FMLP), a synthetic analogue of bacterial peptides, but bovine, porcine and canine neutrophils do not [2-4]. As for cervine cells, however, no information has been available.

As deer farming is on the rise in Japan, comprehensive knowledge of cervine immunity is required for disease control and hygiene management. It seems therefore valuable to understand the characteristics of cervine neutrophils. The purpose of this study was to elucidate the response of cervine neutrophils to various stimuli in comparison with that of human and bovine homologues by evaluating the chemiluminescence, an indicator of the oxidative burst, of the cells.

Six apparently healthy two to three-year-old Sika (Cervus nippon) deer (two intact and two castrated stags and two does) served as blood donors for the cervine studies. Heparinized blood samples were collected through a catheter held in the jugular vein of the animals. Five healthy one-year-old Holstein or Crossbred calves (four female and one castrated) were used as blood donors for the bovine studies. Heparinized blood samples were taken by jugular venipuncture. For the human studies, three healthy adult males were used as blood donors. Neutrophils were isolated from heparinized peripheral blood through single-layer (s.g. 1.077, for cervine and bovine samples) or double-layer (s.g. 1.077 and 1.089, for human samples) density gradient centrifugation and hypotonic lysis following a modification of the previous method [6]. Purity of the neutrophil sample was >95% in each species.

Of four neutrophil stimulants used, phorbol myristate acetate (PMA, Sigma Chemical, St. Louis, U.S.A) and n-formyl-methionyl-leucyl-phenylalanine (FMLP, Sigma Chemical) were each dissolved in dimethyl sulfoxide(DMSO) to a concentration of 1 mg/ml and stored at -20°C as a stock solution. The final concentration for PMA and FMLP in solution with neutrophils was 10 µg/ml (1.6 x 10^4 M) and 43.8 ng/ml (1 x 10^-7 M), respectively.

Cervine (cOZ), bovine (bOZ) and human (hOZ) opsonized zymosans were prepared by mixing zymosan A (Sigma Chemical) with the corresponding fresh serum, respectively, as previously reported [8]. The zymosans were used at a final concentration of 0.5 mg/ml in suspension with neutrophils. Concanavalin A (Boehringer-Mannheim GmbH, Mannheim, Germany) was diluted in Hanks buffered balanced solution (HBSS) to 10 mg/ml and used at a final concentration of 25 µg/ml in neutrophil mixture.

The chemiluminescence assay was performed using a luminescence analyzer (Berthold LUMAT LB9505C, Wildbad, Germany) which evaluates the release of active oxygen during the oxidative burst of the stimulated cells by counting oxygen-transported photons in the presence of luminol. A mixture of 200 µl of HBSS containing 4 x 10^6 neutrophils and 10 µl of luminol solution (10 mg/ml HBSS) were prewarmed at 37°C in the sample chamber and supplied with 10 µl of HBSS containing each stimulant with the appropriate concentration. Photon emission in the mixture was plotted for 40 min. The emission levels were represented as peak values (cpm). All assays were performed in duplicate on the neutrophils and repeated at least three times on the same stimulant. The significance of the difference between mean values was evaluated by Student’s t-test. A level of p<0.05 was regarded as significant.

The results were as follows. OZ, PMA and ConA strongly and consistently stimulated cervine neutrophils as well as bovine and human homologues. As for the cell responses to PMA and ConA, no differences in the peak values or peak times were observed between the species. The response of cervine cells to OZ, however, was relatively selective. When stimulated with homologous cOZ, the cervine neutrophils showed a stronger response with a higher peak value (10^13 cpm vs. 10^9 and 10^7, p<0.05) and a shorter peak time (9.3 min vs. 14.4 and 17.4) than when stimulated with heterologous bOZ or hOZ. FMLP activated human neutrophils. As for cervine and bovine samples, the stimulant did not stimulate them in repeated assays. The cells of both species also failed to respond to the stimulant with a wide range of concentrations from 10^6 to 10^12M (data not shown). A summary of the chemiluminescence response of cervine, bovine and human neutrophils to the stimulants tested is shown in Table 1.
Table 1. A summary of the chemiluminescence response of cervine, bovine and human neutrophils to stimulants

<table>
<thead>
<tr>
<th>Stimulant</th>
<th>Level of neutrophil response</th>
<th>Cervine</th>
<th>Bovine</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMLP</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PMA</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Con A</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Ops. Zym.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Cervine</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>++</td>
</tr>
<tr>
<td>Bovine</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Human</td>
<td>+</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The level was classified, based on peak values, as follows: ; (no peak), + (moderate response with peak values ranging from $10^4$ to $10^5$ cpmp) and ++ (strong response with peak values ranging from $10^5$ to $10^6$ cpmp). ND: Not done.

In the present study, opsonized zymosan strongly and consistently stimulated neutrophil chemiluminescence in all species tested. The results were in good agreement with those of Brown and Roth [1] who have compared bovine and human neutrophil response to the stimulant. Furthermore, the present study revealed that cervine neutrophils have a preference for zymosan opsonized with homologous cervine serum to that treated with heterologous bovine or human serum. As compared with the heterologous sera, the homologous serum might have facilitated the binding of zymosan to cellular surface receptors of cervine neutrophils, thereby accelerating a receptor-mediated signal transduction pathway for the oxidative burst.

PMA and Con A exerted a consistent stimulation on cervine neutrophils as observed on bovine and human cells. PMA is known in human cells to activate the protein kinase C, a trigger of the oxidative burst reaction [10]. As for Con A, however, the present result on bovine neutrophils was different from that of Brown and Roth [1] who did not observe the oxidative burst of the cells in response to lectin. The discrepancy is not well explicable but might have been due to the difference of the concentrations of Con A used in each study.

FMLP has been used as a potent stimulant in human neutrophil studies. However in this study, it was clear that cervine neutrophils, like bovine homologues, do not respond to FMLP. The stimulant has been reported not to stimulate neutrophils from a variety of domesticated species [11]. The failure of the cervine cell response might be due to the lack of FMLP receptors on the cell surface as has been reported in bovine and porcine studies [2, 3].

The chemiluminescence of neutrophils can be used in cattle as a diagnostic aid to detect animals with immunodeficiency [7] or stress-related immunosuppression [5]. Although further studies of cervine neutrophils are required, the present results seem useful, at least in part, toward clinical application of neutrophil chemiluminescence to disease control and hygiene management in deer farming.

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REFERENCES