Clinical Effects of Bovine Lactoferrin on Two Canine Cases with Familial Neutrophil Dysfunction

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ABSTRACT. This study reported detailed clinical effects of bovine lactoferrin on two canine littermates (1 female and 1 male) with familial neutrophil dysfunction and an investigation of their genetic background. Clinical signs caused by severe upper respiratory bacterial infections were observed in these dogs. Oral administration of bovine lactoferrin for a long duration improved their clinical signs (severe uveitis in the female dog and coughing from pneumonia in the male dog). Their backcross dogs that have the same father didn’t show clinical signs of bacterial infection. Neutrophil function tests revealed that the backcross dogs didn’t have any disorders. It is likely that abnormal clinical signs are associated with neutrophil dysfunction in the colony, and the mother dog of these cases might be the genetic carrier of this dysfunction.

KEY WORDS: bovine lactoferrin, familial canine neutrophil dysfunction, genetic background.


Congenital neutrophil dysfunction, which causes defect of neutrophil migration, adhesion, phagocytosis and oxidative killing, has been reported in human patients and several kinds of animals [6, 11, 12]. Leukocyte adhesion deficiency (LAD) is one of these congenital neutrophil dysfunctions and was described more than 25 years ago [20]. Several hundreds of LAD patients were reported on the worldwide scale, showing the failure of innate host defense against bacteria, fungi and other microorganism and impaired wound healing [7]. The children with LAD present the recurrent bacterial infection from infancy and need the long-term administration of antibiotics [2].

On the other hand, canine cases of LAD were reported as canine leukocyte adhesion deficiency (CLAD) in several pure breed dogs [5, 9, 21]. Sequence analysis of neutrophil CD18 alleles had been identified a single nucleotide G-to-C transversion at position 107, which lead to replacement of cysteine by serine at residue 36 in CLAD cases [8, 12]. We previously reported a new type of neutrophil dysfunction in mixed breed canine littermates. The neutrophil dysfunction was caused by decreased transcriptional level of β2-integrin without mutation, leading to down-regulation of CD18 expression [14]. The dogs showed multiple defects of adhesion-related neutrophil functions and consequently recurrent bacterial infections from puppyhood [14].

The therapy of genetic neutrophil dysfunction is very difficult, and there is no specific remedy. The patients of LAD and CLAD are always treated by long-term administration of antibiotics for recurrent bacterial infection and other symptomatic treatment, however, would not obtain complete improvement. Two canine littermates with neutrophil dysfunction showed that their neutrophil functions were upregulated by administration of bovine lactoferrin in a previous study [13]. This study reported detailed clinical effects of oral administration of bovine lactoferrin on the same littermates with neutrophil dysfunction and an investigation of their genetic background.

Two mixed-breed canine littermates with familial neutrophil dysfunction were referred to our Veterinary Teaching Hospital of Iwate University for intractable bacterial infection. The female dog was presented at the age of 9 months. Mucopurulent mucus caused by rhinotracheitis had been observed from 3 months old. Binocular mucopurulent eye discharge and subsequent uveitis with a progressive course were seen. The female dog rubbed her eyes, followed by hemorrhage and deteriorating vision. The clinical signs relapsed promptly after temporary improvement by treatment with antibiotics and interferon-γ. At the first medical examination in our hospital, body weight of the female dog was 8.15 kg (body condition score, 2/5), and the dog showed lethargy, anorexia and pyrexia (39.4°C). The major clinical features were severe purulent conjunctivitis, rhinitis, bilateral uveitis, occasional epistaxis and pneumonia with productive cough (Fig. 1A). Blood examination revealed hypoalbuminemia (1.42 g/dl) and hyperglobulinemia (4.98 g/dl). White blood cell count (WBC) and neutrophil count were 14,000/µl and 7,000/µl, respectively. The results of other blood examination were unremarkable. Serum anti-distemper virus, anti-adenovirus 1 and anti-adenovirus 2 antibodies were negative. For first 2 weeks, the female dog...
was treated with cephalexin (30 mg/kg, po, bid), dropping lotions (0.3% norfloxacin and 0.1% dexamethasone sodium metasulfobenzoate) and inhalation therapy. However, clinical improvement was not observed. There were nausea, anorexia, cough and severe mucopurulent nasal discharge with dyspnea during the period.

On the other hand, the male dog was presented to our hospital for intractable purulent inflammation of upper respiratory tract 6 years after the first visit of female dog. The dog had developed similar clinical symptoms of bacterial infection identical to the female littermate, but the symptoms had been mild during puppyhood. Physical examination at the first visit in our hospital revealed severe mucopurulent eye discharge, conjunctivitis, scleritis and bilateral corneal opacity (Fig. 1B). The male dog showed poor physical condition (body weight: 10.4 kg, body condition score: 2/5), and anorexia. Severe productive cough was observed, and radiography examination revealed the sign of severe pneumonia (Fig. 2A and 2B). Cytological examination of eye discharge and bronchial lavage fluid revealed purulent inflammation with many segmented neutrophils and bacteria, Pasteurella canis and Gram-negative bacilli. Shown in Table 1, hematological examination showed leukocytosis (WBC: 22,600/µl, neutrophils: 18,532/µl), macrocytic hypochromic anemia (mean corpuscular volume; MCV: 84.5 fl, mean corpuscular hemoglobin concentration; MCHC: 28.8 g/dl), hypoalbuminemia (1.35 g/dl) and hyperglobulinemia (5.05 g/dl). Coombs test and antinuclear antibody were negative. The results of other blood examination were unremarkable. During the first 2 weeks, the male dog was treated with antibiotics administration based on antibiotic sensitivity test (ampicillin sodium, 30 mg/kg, iv, tid; orbifloxacin, 5 mg/kg, sc, sid), fluid therapy (lactate Ringer’s solution, aminophylline, vitamins) and inhalation therapy. There was a poor response of these treatments and still anorexia, productive cough, mucopurulent discharge of eyes and nose seen in the male dog.

Our previous study reported that oral administration of bovine lactoferrin, which is 80 kDa iron-binding glycoprotein isolated from bovine milk increased neutrophil phagocytic activity in FIV-infected cats and ameliorated their intractable stomatitis [18]. The female and male dogs were treated with bovine lactoferrin internally (Morinaga Milk Industry Company., Tokyo, Japan, 40 mg/kg, sid) in combination with the other symptomatic therapy including antibiotics therapy for a month and 140 days, respectively. Oral treatment with bovine lactoferrin increased the neutrophil functions [13], followed by improvement of the clinical signs and hematologi-
Fig. 2. The lateral (A) and ventrodorsal thoracic radiograph (B) showing pneumonia with a diffuse alveolar pattern in the male dog with neutrophil dysfunction at the first medical examination in our hospital. The improvement of diffuse alveolar pattern was observed 140 days after treatment with bovine lactoferrin, resulting in disappearance of cough and aerophagia (C and D).

Table 1. The findings of hematological and physical examinations in the male dog with neutrophil dysfunction

<table>
<thead>
<tr>
<th>Oral lactoferrin-administration</th>
<th>Pretreatment</th>
<th>14 days</th>
<th>28 days</th>
<th>94 days</th>
<th>140 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (/$\mu l$)</td>
<td>22,600</td>
<td>16,000</td>
<td>31,700</td>
<td>23,200</td>
<td>15,800</td>
</tr>
<tr>
<td>Neutro. (/$\mu l$)</td>
<td>18,532</td>
<td>11,000</td>
<td>27,400</td>
<td>17,600</td>
<td>10,428</td>
</tr>
<tr>
<td>RBC (×10$^6$ /$\mu l$)</td>
<td>296</td>
<td>346</td>
<td>313</td>
<td>512</td>
<td>529</td>
</tr>
<tr>
<td>Hb. (g/dl)</td>
<td>7.2</td>
<td>8.2</td>
<td>7.6</td>
<td>9.6</td>
<td>10.4</td>
</tr>
<tr>
<td>Ht. (%)</td>
<td>25.0</td>
<td>30.0</td>
<td>28.0</td>
<td>34.5</td>
<td>37.0</td>
</tr>
<tr>
<td>Alb. (g/dl)</td>
<td>1.35</td>
<td>1.38</td>
<td>2.23</td>
<td>2.55</td>
<td>3.09</td>
</tr>
<tr>
<td>α-glob. (g/dl)</td>
<td>1.51</td>
<td>1.43</td>
<td>ND</td>
<td>1.11</td>
<td>ND</td>
</tr>
<tr>
<td>β-glob. (g/dl)</td>
<td>2.35</td>
<td>1.94</td>
<td>ND</td>
<td>1.59</td>
<td>ND</td>
</tr>
<tr>
<td>γ-glob. (g/dl)</td>
<td>1.19</td>
<td>1.64</td>
<td>ND</td>
<td>0.95</td>
<td>ND</td>
</tr>
<tr>
<td>A/G</td>
<td>0.27</td>
<td>0.27</td>
<td>ND</td>
<td>0.70</td>
<td>ND</td>
</tr>
<tr>
<td>Body weight (Kg)</td>
<td>10.4</td>
<td>9.25</td>
<td>9.8</td>
<td>11.15</td>
<td>11.45</td>
</tr>
<tr>
<td>Body temperature (°C)</td>
<td>39.2</td>
<td>ND</td>
<td>38.2</td>
<td>39.3</td>
<td>38.6</td>
</tr>
</tbody>
</table>

Pretreatment: at the first medical examination in our hospital.
ND: Not determined.
ical findings. In the female dog with neutrophil dysfunction, the clinical signs of upper respiratory bacterial infection and corneal opacity were gradually improved two weeks after from oral lactoferrin administration. One month after the beginning of lactoferrin treatment, conjunctivitis, uveitis and rhinitis almost disappeared (Fig. 1C). The combined use of lactoferrin was potent in improving those clinical signs compared to single antibiotic therapy. After that, the female dog was treated with bovine lactoferrin and antibiotics every time when her purulent inflammation occurred.

In the male dog with neutrophil dysfunction, the remarkable amelioration was not observed during 2 weeks of lactoferrin administration, while the dog showed slowly recovery of appetite and normal body temperature. In addition, there were the slight decreases of both mucopurulent eye discharge and exudative cough. Twenty-eight days after lactoferrin treatment, exudative cough and oculo-nasal mucopurulent discharge disappeared, and corneal opacity was gradually ameliorated (Fig. 1D). The male dog showed good recovery and body weight gain (11.15 kg), and the nasal obstruction and pneumonia were relieved on 94-day of lactoferrin treatment (Fig. 2C and 2D). Thereafter, the male dog couldn’t take lactoferrin for 2 weeks because of the owner’s reason, inducing a recurrence of mild oculo-nasal mucopurulent discharge on 140-day of lactoferrin treatment. As it is shown in Table 1, WBC was gradually decreased and serum albumin charge on 140-day of lactoferrin treatment. As it is shown in Table 1, WBC was gradually decreased and serum albumin charge on 140-day of lactoferrin treatment. In the female dog with neutrophil dysfunction, the clinical signs of upper respiratory bacterial infection and corneal opacity were gradually improved two weeks after from oral lactoferrin administration. One month after the beginning of lactoferrin treatment, conjunctivitis, uveitis and rhinitis almost disappeared (Fig. 1C). The combined use of lactoferrin was potent in improving those clinical signs compared to single antibiotic therapy. After that, the female dog was treated with bovine lactoferrin and antibiotics every time when her purulent inflammation occurred.

The neutrophil dysfunction seen in the littermates suggested genetic relationship in our previous study [14]. In order to examine their genetic background, their pedigree was confirmed by hearing investigation from the owner (Fig. 3). Their mother dog (mixed-breed) had died at the age of 9. Their father dog (mixed-breed) is still alive, but we could not have access to the dog. The parents didn’t have any clinical signs of bacterial infection. They had 5 puppies (2 females and 3 males) in 2,000 including the 2 littermates with neutrophil dysfunction. The other 3 littermates have no clinical signs. There was inbreeding in the colony. One of female littermates without clinical signs crossed with their father dog and bred 4 puppies. The backcross puppies showed no clinical signs of bacterial infection. Hematological examination and neutrophil function tests were performed in these 4 puppies and 4 healthy puppies as the controls. Hematological examination showed that WBC and neutrophils in 4 healthy puppies (a female and 3 males, 2 months old) were 13,500 ± 1,183/µl and 7,229 ± 1,978/µl, respectively, while WBC and neutrophils in the 4 backcross puppies (3 females and a male, 2.5 months old) were 11,950 ± 1,763/µl and 6,542 ± 1,000/µl, respectively (mean±S.D.). There was no abnormal finding of hematological examination in the backcross puppies.

Neutrophil function tests were performed by using the same methods as described in our previous study [14]. Statistical differences were evaluated by the paired t-test, and a P value less than 0.05 was considered significant. Neutrophil superoxide-production of the backcross dogs was evaluated by using luminol-dependent chemiluminescence response in order to clarify whether neutrophil dysfunction existed in the backcross dogs or not. The isolated peripheral neutrophils (5 × 10^8 cells) by centrifugation with Ficoll-Conray solution (density, 1.077), 700 µl HBSS and 100 µl of 100 µM luminol were mixed. The mixture was activated by adding 200 µl serum-opsonized zymosan (5 mg/ml) and chemiluminescence was measured with the luminometer (Luminescencer PSN, ATTO, Tokyo, Japan). Shown in Fig. 4A, luminol-dependent chemiluminescence response of neutrophils from all backcross puppies tended to increase (peak height, 136,454 ± 38,755/sec) compared to that of normal puppies (815 ± 79 sec). The delay of peak time of the response in the backcross puppies (669 ± 33 sec) was not observed compared to that of normal puppies (815 ± 79 sec). The curve of luminol-dependent chemiluminescence response was normal in the backcross puppies compared with controls. The backcross puppies in neutrophil adherence to nylon fibers exhibited the same levels, 26.28 ± 6.91%, compared with healthy puppies, 23.51 ± 2.63% (Fig. 4B). Neutrophil nonspecific phagocytosis of fluorescent microspheres, which was measured by flow cytometric method, in the backcross puppies and healthy puppies was 37.65 ± 5.73% and 51.72 ± 15.39%, respectively (Fig. 4B). Neutrophil CD11b/CD18 expression in backcross puppies was analyzed by the whole blood flow cytometric method, because the male dog with neutrophil dysfunction presented a decrease in the expression of CD11b/CD18 [14]. Briefly, after staining with FITC-labeled anti-CD11b and CD18, cells were resuspended in 0.5% paraformaldehyde in PBS and analyzed by flow cytometer (FACScan, Becton Dickinson, Tokyo, Japan). The expression of CD11b/CD18 was higher than that of healthy puppies (Fig. 4C). The neutrophil surface expression of CD11b in backcross puppies and healthy puppies was 16.93 ± 4.83% and 10.38 ± 3.71, respectively. Expression of CD18 in backcross puppies was higher (73.13 ± 2.79%) than that
There were no abnormal observations of all neutrophil function tests on individualization of implementation of the backcross puppies. Therefore, this observation suggested that the disorders of neutrophil function didn’t exist in all backcross puppies compared to 2 dogs with neutrophil dysfunction as described in a previous study [14].

This study reported an investigation of their genetic background and detailed clinical effects of bovine lactoferrin on 2 canine littermates with familial neutrophil dysfunction. The neutrophil dysfunction of the littermates suggested genetic relationship, because of their medical history of recurrent bacterial infections from puppyhood. Hearing investigation from the owner revealed the inbreeding in the colony. Neutrophil function tests showed that the backcross dogs, which have the same father as our 2 cases, didn’t have any disorders. Therefore, it is likely that abnormal clinical signs are associated with neutrophil dysfunction in the colony, and the mother dog of the 2 littermates with neutrophil dysfunction might be the genetic carrier of this disease.

This study revealed that oral administration of bovine lactoferrin for a long duration improved the clinical signs of severe bacterial infection in 2 littermates with familial neutrophil dysfunction. Clinical signs in the dogs with neutrophil dysfunction were manifested shortly after birth, with development of rhinotracheitis and ophthalmia with fever, followed by recurrent, severe bacterial infections. Their major clinical features were frequent and progressive mucopurulent oculo-nasal inflammation with bacterial overgrowth, pneumonia and pyrexia. The clinical history was consistent with findings in Irish Setters with CLAD and BLAD-affected cattle [10]. Hematological studies in CLAD or BLAD affected animals showed marked persistent leukocytosis with neutrophils constituting up to 90% of the leukocytes in peripheral blood (>500,000/µl in CLAD, >80,000/µl in BLAD) [10].

The severe neutrophilia was not observed in our canine cases with neutrophil dysfunction. Hyperglobulinemia seen in our canine cases suggested frequent and severe bacterial infectious episodes in the past. In addition, our 2 cases could grow up to adult age, while most CLAD-affected dogs typically die from bacterial infectious complications by six months of age [10]. These findings may suggest that this type of neutrophil dysfunction in our 2 dogs would present symptoms of lesser intensity than CLAD.

A prolonged course of antibiotics therapy represents the 1st line of therapy at the onset of clinical signs of CLAD- or BLAD-affected animals [4, 10]. Several studies reported that some of antibiotics or granulocyte colony-stimulating factor had an inhibitory effect on neutrophil functions [16, 17, 19]. Death usually ensures from severe bacterial infections in most animals with CLAD or BLAD. Recently, stem cell gene therapy for genetic neutrophil dysfunction has been reported in CLAD [1, 3]. However, the therapy option is not available immediately to apply in a clinical setting, because there are many problems that should be solved till it comes to be applied in the clinical cases, e.g. the adverse effects or high cost medical care. Therefore, at present, there were the limited treatment options available for cases with congenital...
neutrophil dysfunction. Treatments for our dogs with neutrophil dysfunction were similar to those for dogs with CLAD. However, symptomatic treatment alone didn’t improve the clinical signs of severe bacterial infections and hematological findings in the dogs with neutrophil dysfunction. Thus, the cases with familial neutrophil dysfunction needed for a treatment that could modulate the neutrophil functions without adverse effects for long-term administration. Lactoferrin is one of key factors in primary host defense system against infection [22]. We previously reported that oral administration of bovine lactoferrin enhanced neutrophil phagocytic activity in FIV-infected cats [18]. We also observed that oral treatment with bovine lactoferrin modulated neutrophil functions in healthy dogs and cats. In addition, oral administration of bovine lactoferrin has shown to increase phagocytic activity and superoxide production or decrease adherence of peripheral neutrophils in healthy volunteers [24]. It has also been demonstrated that exogenous lactoferrin has various biological functions, including anti-inflammatory and immunomodulatory activities [15, 23]. In this study, oral administration of bovine lactoferrin in combination with the other symptomatic therapy improved clinical signs of infections in the 2 canine littermates with neutrophil dysfunction. Concurrently, the markers of inflammation in hematological examination were gradually decreased on the male dog in this study after treatment with lactoferrin. Judging from these findings and our previous study [13], oral administration with bovine lactoferrin for a long duration seems to bring the improvement of the clinical features such as severe rhinotraceitis, ophthalmia and pneumonia by increasing the neutrophil functions of these dogs. Further studies are required, but it suggests that oral administration of bovine lactoferrin may have a potential as one of treatment options for neutrophil dysfunction without unexpected secondary effects.

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