Assessment of CD11b and CD11/18 integrin expression on leukocytes of dogs with atopic dermatitis

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Abstract

Cytometric analysis was performed in order to detect the expression of surface molecules CD11b and CD11/18 on lymphocytes, granulocytes, and monocytes of peripheral blood of dogs with atopic dermatitis (AD) complicated by purulent dermatitis. The percentage of peripheral blood lymphocytes with CD11b and CD11/18 was found to be significantly higher (P < 0.05) in dogs with AD than in healthy dogs. The percentage of granulocytes expressing CD11b molecule was significantly lower in dogs with complicated AD compared to healthy dogs and dogs with uncomplicated AD. The expression of CD11b and CD11/18 integrin on monocytes of dogs with complicated AD depended on the severity of symptoms and was higher in dogs with deep purulent dermatitis. It was concluded that the expression of CD11b and CD11/18 integrin on leukocytes of dogs with AD depends on the stage of the disease.

Key words: dogs, atopic dermatitis, integrins, CD11b, CD11/18, flow cytometry.

Introduction

Cell adhesion molecules (CAMs), including integrins, are cell membrane glycoproteins, which serve as receptor proteins (1, 2, 20). Integrins on the surface of activated leukocytes are responsible for interactions between cells and extracellular matrix, as well as among leukocytes during adhesion (5, 16, 20). The type of stimulating factor, activation of different signal transduction pathways, and type of extracellular cell matrix (ECM) determine the specific activity of integrins (20). β2 integrins (CD18), which are present on leukocyte membranes bind to the ICAM group and are responsible for adhesion of leukocytes to the endothelium during inflammation. They combine with αL, αM, and αX integrin subunits to form CD11a/CD18 (LFA-1), CD11b/CD18 (Mac-1, CR3), and CD11c/CD18 (gp150/95, CR4), respectively (1, 3, 10, 15). The CD11a/18 integrin mainly occurs on lymphocytes and is responsible for adhesion of T cells to antigen-presenting cells and of leukocytes to endothelial cells. The CD11b/18 integrin, similarly to CD11c/18, is expressed on neutrophils, monocytes, macrophages, and natural killer cells (NK) cells. These integrins are mainly responsible for adhesion of neutrophils to endothelial cells, their passage through the blood vessel wall, and phagocytosis of immune complexes and microorganisms (1, 13). The CD11b/18 receptor dysfunction has been demonstrated in peripheral blood neutrophils of dogs with chronic purulent dermatitis and in Irish Setters with leukocyte adhesion deficiency (LAD) (6, 19).

The aim of the study was cytometric analysis of the expression of surface molecules CD11b and CD11/18 on leukocytes of peripheral blood of dogs with atopic dermatitis complicated by purulent dermatitis.

Material and Methods

Animals. The study was conducted on two experimental groups of dogs (group IB and IIB) and on the control group (group C). Group IB consisted of 24 females and 22 males of different breeds, aged 1.5 to 3.5 years with atopic dermatitis (AD) complicated by purulent inflammation of the skin. Group IIB included 18 females and 24 males of different breeds, aged...
1.3-3.2 years with uncomplicated AD (stage of pruritic erythematous disease) previously treated with cephalosporin (Kefavet vet.-Orion Corp.) at a dose of 30 mg/kg b.w./d. Shampoo treatment and monodiет were used in accordance with the applicable rules, if required. The experimental dogs fulfilled the clinical criteria for the diagnosis of AD (4, 11, 12, 17). The diagnosis of AD was based on disease history, clinical examination, and results of intradermal tests performed with an Artuvef Test set (Artu Biologics Europe BV, Netherlands). Intradermal test results showed polyvalent allergy and positive reactions to house dust mites in all experimental dogs. Animals of both experimental groups were not treated with corticosteroids, calcineurin inhibitors, and/or antihistamines for the last 2 months. Laboratory dermatological tests were performed according to the accepted rules, and their selection depended on the patient's clinical status (4, 11). The results excluded dermatophytosis and ectoparasites: fleas, lice, mallophaga or mites (Demodex sp., Sarcoptes sp., Cheyletiella sp., and others). Cytological studies in dogs of group IB confirmed surface, superficial, or deep purulent dermatitis (pyoderma) caused by Gram-positive Staphylococcus sp. or excluded the bacterial skin infection. The control group (C) consisted of clinically healthy dogs (five females and five males) of mixed breeds, aged 1–3 years. The dogs were dewormed and vaccinated against canine distemper, parvovirus, and rabies. The results of their dermatological and intradermal tests were negative.

Cytometric studies. Flow cytometry was used to measure the percentage of blood cells expressing CD11b and CD11/18 molecules. Blood was collected from the lateral saphenous or cephalic vein to chemically pure and sterile glass tubes with heparin sodium (Biochemie GmbH, Austria), 5U/1 mL of blood. Fluorochrome-labelled Rat Anti Canine monoclonal antibodies directed against antigens present on the surface of peripheral blood leukocytes were used for cytometric analysis of CD11b/18 molecules (MCA1040F, AbD Serotec), Fluorochrome-labelled (MCA1777S, AbD Serotec) CD11b Mouse Anti-Canine monoclonal antibodies directed against surface antigens present on canine peripheral blood leukocytes and secondary polyclonal RABBIT F (ab’)-2 Anti-Mouse IgG RPE (AbD Serotec) were applied to identify CD11b molecules. Cytometric analysis was performed using the Epics XL flow cytometer (Epics XL flow cytometer Beckman - Coulter, Comesa CH-4016, Switzerland) according to the manufacturer’s recommendations at the Department of Epizootiology and Clinic of Infectious Diseases, University of Life Sciences in Lublin. Prior to determinations, quality control and autostandardisation were carried out using Coulter Flow-Check, Coulter Flow-Set Compensation Reagents. The half-life rate of variety (HPCV) was verified with the expected value to check the stability of optical and flow systems. Using the Flow-Set preparation, Quantum TM R-PE MESF (ABD Serotec catalogue number 827A) and Quantum TM FITC MESF (AbD Serotec catalogue number 825A), light scattering and fluorescence intensity were standardised by adjusting the voltage and enhancement to a particular mean value based on the control application. Moreover, suitable reagents were used to determine the colour levelling system.

Statistical analysis of results was carried out using the Mann-Whitney U test for nonparametric data, and correlations were analysed using the Spearman’s rank correlation test for P < 0.05.

Results

The results obtained in IB, IIB, and control (C) groups are presented in Table 1. The percentage of peripheral blood lymphocytes showing the presence of CD11b and CD11/18 molecules was found to be significantly higher (P < 0.05) in dogs with AD than in healthy dogs. In dogs with complicated AD (group IB), the average percentage of CD11b lymphocytes was 64.35%, compared to dogs with uncomplicated AD (group IIB) 50.46% and healthy animals (group C) 43.57%.

<table>
<thead>
<tr>
<th>Cells</th>
<th>Adhesion molecules</th>
<th>Percentage of leukocytes with integrin receptors (X ± SD)</th>
<th>Group IB (n = 46)</th>
<th>Group IIB (n = 42)</th>
<th>Group C (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes</td>
<td>CD11b</td>
<td>64.35 ± 14.58* (P = 0.003)</td>
<td>50.46 ± 7.32*</td>
<td>43.57 ± 1.61*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CD11/18</td>
<td>77.46 ± 20.33* (P = 0.008)</td>
<td>61.34 ± 7.33*</td>
<td>54.26 ± 2.38*</td>
<td></td>
</tr>
<tr>
<td>Granulocytes</td>
<td>CD11b</td>
<td>77.25 ± 15.23* (P = 0.001)</td>
<td>90.21 ± 8.68</td>
<td>92.78 ± 3.94*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CD11/18</td>
<td>12.71 ± 5.97</td>
<td>11.18 ± 7.12</td>
<td>12.56 ± 3.94*</td>
<td></td>
</tr>
<tr>
<td>Monocytes</td>
<td>CD11b</td>
<td>61.17 ± 13.57</td>
<td>54.61 ± 12.46</td>
<td>59.67 ± 7.98</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CD11/18</td>
<td>44.46 ± 15.58</td>
<td>46.56 ± 12.53</td>
<td>48.62 ± 8.83</td>
<td></td>
</tr>
</tbody>
</table>

Groups: IB - dogs with complicated AD, IIB - dogs with uncomplicated AD, C - controls. A mean (X), standard deviation (SD), and significance level (P) in relation to Group C. * - statistically significant inter-group differences found using the Mann-Whitney U test for nonparametric data.

Table 1. The mean percentage (x ± SD) of CD11b and CD11/18 integrin expressed on peripheral blood leukocytes of dogs with complicated and uncomplicated AD measured by flow cytometry.
The percentage of CD11/18 lymphocytes was 77.46% in dogs with complicated AD, 61.34% in those with uncomplicated AD, and 54.26% in healthy animals. The peripheral blood granulocytes showed the presence of CD11b molecules both in dogs with atopic dermatitis and in healthy ones. The percentage of granulocytes with CD11b molecules was significantly lower in dogs with complicated AD (77.25%) than in healthy dogs (92.78%) and in dogs with atopic dermatitis in the erythematous-pruritic stage (90.21%). However, the percentages of CD11b granulocytes and CD11b, CD11/18 monocytes in dogs with AD and healthy dogs were similar (Table 1). In group IB (with complicated AD), the Spearmann’s method revealed a significant negative correlation (R = -0.30) between the percentage of CD11b molecules on monocytes and granulocytes (Fig. 1) and between CD11/18 and CD11b molecules (R = -0.37) on granulocytes (Fig. 2).

Moreover, the percentage of CD11/18 molecules on monocytes of peripheral blood in IB dogs with deep purulent dermatitis (56.42, on average) was significantly higher (P < 0.05) compared to animals with superficial/surface purulent dermatitis (36.44%) (Table 2). The percentages of the remaining parameters in this group were comparable, irrespective of the stage of inflammation.

Fig. 1. The negative correlation between the percentage of CD11b molecules on granulocytes and monocytes (R = -0.30) in dogs with AD complicated by purulent inflammation. Spearman rang test (P < 0.05)

Fig. 2. The correlation between the percentage of CD11b and CD11/18 molecules on granulocytes (R = -0.37) in dogs with AD complicated by purulent inflammation. Spearman rang test (P < 0.05)

Fig. 3. The correlation between the percentage of CD11b molecules on monocytes and granulocytes (R = -0.41) in dogs with AD complicated by surface/superficial purulent inflammation. Spearman rang test (P < 0.05)

Fig. 4. The correlation between the percentage of CD11b and CD11/18 molecules on lymphocytes (R = -0.51) in dogs with AD complicated by deep purulent inflammation. Spearman rang test (P < 0.05)

Fig. 5. The correlation between the percentage of CD11b and CD11/18 molecules on granulocytes (R = -0.62) in dogs with AD complicated by deep purulent inflammation. Spearman rang test (P < 0.05)
Table 2. The mean percentage of peripheral blood leukocytes of dogs with complicated AD expressing CD11b and CD11/18 integrins according to the extent of inflammation (X±SD) measured by flow cytometry

<table>
<thead>
<tr>
<th>Cells</th>
<th>Adhesion molecules</th>
<th>Percentage of integrin receptors (X±SD) in dogs with AD complicated by purulent dermatitis</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Surface/superficial pyoderma (n = 28)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Deep pyoderma (n = 18)</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>CD11b</td>
<td>61.59±1.42</td>
<td>0.1903</td>
</tr>
<tr>
<td></td>
<td>CD11/18</td>
<td>76.25±21.32</td>
<td>0.8257</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>CD11b</td>
<td>77.35±14.83</td>
<td>0.9630</td>
</tr>
<tr>
<td></td>
<td>CD11/18</td>
<td>11.74±5.76</td>
<td>0.2237</td>
</tr>
<tr>
<td>Monocytes</td>
<td>CD11b</td>
<td>61.69±14.94</td>
<td>0.9630</td>
</tr>
<tr>
<td></td>
<td>CD11/18</td>
<td>36.44±13.85*</td>
<td>0.0000</td>
</tr>
</tbody>
</table>

*- statistically significant inter-group differences demonstrated using the Mann-Whitney U test for nonparametric data

In dogs with AD complicated by surface/superficial purulent dermatitis, a significant (P < 0.05) negative correlation (Spearman’s methods) was demonstrated (R=-0.41) between the percentage of CD11b molecules on monocytes and granulocytes (Fig. 3), whereas in dogs with AD complicated by deep purulent dermatitis, such a correlation was observed between the percentage of CD11b and CD11/18 molecules on lymphocytes (R=-0.51) and granulocytes (R=-0.62) (Figs 4 and 5).

Discussion

This study aimed to compare the expression of CD11b and CD11/18 (β2) integrins on leukocytes of dogs with complicated and uncomplicated AD, as well as in healthy dogs. In the literature, there are only a few reports devoted to characteristics of CD11b and CD11/18 molecules on lymphocytes, monocytes, and granulocytes of healthy dogs, or evaluation of their activity in purulent dermatitis (8, 13, 14, 18). Moreover, there is no data on involvement of these integrins in AD, which makes the comparison of the obtained results difficult. According to the received findings, the expression of CD11b and CD11/18 integrins on the surface of lymphocytes was significantly higher in experimental groups than in the control group. This indicates that CD11b and CD11/18 integrins in dogs with AD are essential for the transmigration of immunocompetent cells through the vessel wall and their targeted migration to the source of ongoing skin inflammation. The increased β2 integrin expression on lymphocytes found in dogs with AD, regardless of the stage of disease, is likely to implicate their important role in the pathogenesis of the disease. In the studies on healthy humans, Kawai et al. (7) have demonstrated the presence of CD11b integrins on peripheral blood B cells suggesting their key role in the migration of B cells and immunological memory cells. Żebrowska et al. (20) have shown that β2 integrins determine the interaction between keratinocytes and T lymphocytes present in the skin. Moreover, studies on the pathomechanism of subepidermal bullous disease performed by Liu et al. (9) on a mouse model have revealed that blockade of β2 integrin activity inhibits the disease development. Thus, a significant increase in the percentage of CD11b and CD11/18 integrins on lymphocytes of dogs with AD may indicate their involvement in the development of the pruritic erythematous stage of disease. In contrast, a significant decrease in CD11b integrins on granulocytes in dogs with atopic dermatitis complicated by purulent dermatitis compared to healthy animals, and dogs with uncomplicated AD reveals a lack of essential involvement of these cells in the pathogenesis of disease. However, it cannot be ruled out that dogs with complicated atopic dermatitis develop disorders of granulocyte adhesion, as indicated by the data obtained in the group with uncomplicated AD. Furthermore, the findings demonstrate that the expression of β2 integrins on monocytes in dogs with complicated AD depended on the severity of symptoms, and was higher in dogs with deep purulent dermatitis. Taking into account that in dogs with AD deep pyoderma is chronic and results from surface or superficial inflammation, increased percentages of monocytes with β2 integrins found in the study indicate their involvement in phagocytosis and cytotoxicity of staphylococcal infection (1, 19). Moreover, the results demonstrate a high percentage of granulocytes with CD11b integrins in healthy dogs, which confirms the data reported by Ruaux and Williams (13). Surface receptors of CD11b and CD11/18 integrins present on granulocytes are considered to be the indicators of adhesion and migration of these cells during inflammation (13, 19). Hence, a significant decrease in CD11b integrins on granulocytes of dogs with AD complicated by purulent skin inflammation observed in the study indicates the impaired phagocytic activity.

The results of the study have demonstrated that percentages of CD11b and CD11/18 integrins expressed on leukocytes in dogs with AD depend on the stage of disease; however, a further insightful research is required to explain their role in the pathogenesis of canine atopic dermatitis.

Acknowledgments. The study, approved by the Local Animal Experimentation Ethics Committee, was supported by funding of the Ministry of Science and Higher Education (project No. N30801632/1409).
References