Expression of CD3, CD4, CD8, CD21, and MHC II lymphocyte antigens and serum IL-10 concentration in dogs with atopic dermatitis complicated by purulent dermatitis

Iwona Taszkun

Sub-department of Clinical Diagnostic and Veterinary Dermatology, Department and Clinic of Internal Medicine, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, 20-612 Lublin, Poland, iwona.taszkun@up.lublin.pl

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Abstract

Dog blood samples were analysed cytometrically and immunoenzymatically. In dogs with atopic dermatitis complicated by purulent dermatitis, the percentage of lymphocytes with CD8, CD21 antigens, as well as IL-10 concentration in serum were significantly higher than in healthy dogs. The results indicate that immunosuppression induced by the activity of an infectious agent plays an important role at this stage of the disease. This thesis was further confirmed by a high positive correlation between MHC II CD3, CD4, and CD21 expression and a negative correlation between cells with MHC II and CD8 lymphocytes.

Key words: dogs, canine atopic dermatitis, lymphocyte subpopulations, IL-10, flow cytometry.

Introduction

Canine atopic dermatitis (AD, eczema, atopic canine disease, allergic inhalant dermatitis) is a pruritic and inflammatory allergic skin disease. The disease is featured by an increase in IgE antibodies against environmental allergens (1, 12, 14, 16). Dogs with AD have a tendency to purulent inflammation of the skin, Malassezia dermatitis, and/or kerato-seborrhoeic syndrome (13, 14, 17). Nowadays, an increasing number of researchers believe that the pathogenesis of AD in dogs is due to comorbidity in the immune system leading to the development of type I hypersensitivity (Gell-Coombs classification), dysfunction of the epidermal barrier, and disorders of skin colonisation by microorganisms such as bacteria and fungi (7, 12, 13, 17, 18).

Lymphocytes, the primary cells of the immune system, regulate and enhance the immune processes and are actively involved in the effector phase of the response. This is caused by a series of interactions between the antigen presenting cells (APC) and the effector cells, namely lymphocytes. Specific activity of lymphocytes is assessed by identifying CD (cluster of differentiation) molecules present on the cell surface. T cells with CD3 molecules are responsible for the transmission of signals to the cell interior (5, 9). This determines the recognition of antigens presented by dendritic cells (Langerhan's cells in the skin) by molecules of the major histocompatibility complex (MHC). T lymphocytes exhibiting the presence of CD4 cell surface receptors can promote and induce both humoral (Th2 cells) and cellular (Th1) immune response. The indicator of the humoral immune response activity is the production of immunoglobulins by B cells (CD21) as well as an increase in serum levels of interleukin 10 (9, 10). Interleukin 10 (IL-10), known as an immunosuppressive and anti-inflammatory cytokine, is produced primarily by Th2 lymphocytes, but also by B lymphocytes, monocytes, macrophages, and keratynocytes. Its activity inhibits the synthesis of inflammatory cytokines, reduces the molecular expression of MHC class II antigens on APC, inhibits the formation of Th1 cells, and stimulates “directed” B cells to switch to IgE synthesis (10, 19). It is believed that the Th2 immunological response is...
controlled by CD4⁺CD25⁺ T regulatory lymphocytes (Treg) and regulatory T cells type I able to release IL-10 (1). However, T cells indicating the presence of CD8 molecule play a cytotoxic/suppressor role as Te/Ts lymphocytes. It is believed that CD8 lymphocytes inhibit immune response (9). The mechanism of their action is based on the ability to react with presented antigen both at the participation of molecules of the MHC class II as well as MHC class I.

Recent data indicates that in the course of AD, CD4 lymphocytes (both Th2 and Th1) and CD8 lymphocytes are involved (17). It is known that in acute AD the activation of B cells (CD21) in the immune response plays a dominant role, which leads to the synthesis of specific IgE. However, in the chronic course of the disease the stimulation of Th1 cells responsible for the development of cell-mediated response is observed (1, 5, 17).

The aim of the study was to evaluate subpopulations of cells expressing MHC II, CD3, CD4, CD8, and CD21 antigens in lymphocytes and IL-10 concentration in the peripheral blood of young dogs suffering from AD complicated by purulent inflammation of the skin, and analysis of their relationship.

Material and Method

Animals. The experimental group consisted of 51 dogs (27 non-pregnant females and 24 males of various breeds), aged from 1.5 to 3.5 years (average age was 2.5 years), with AD complicated by purulent dermatitis. The percentage of specific breeds of the dogs was as follows: French and English bulldog (27.45%), American Staffordshire terrier (23.53%), boxer (23.53%), Dalmatian (9.81%), Golden retriever (5.88%), German shepherd (3.92%), and others (5.88%).

The dogs qualified to the test fulfilled the clinical criteria allowing the identification of AD (7, 16). The diagnosis of AD was made on the basis of history, physical examination, and intradermal test results using the Artuvetrin Test Set (Artu Biologicals Europe BV, Netherlands). Intradermal test results showed multi-validity of allergies (polyvalency) in all dogs from the experimental group. The animals were not treated with glucocorticoids, calcineurin inhibitors, antihistamines, or antibiotics for the last 2 months. Laboratory dermatological tests were performed in accordance with applicable rules, and their selection depended on the patient's clinical status (7). The results excluded dermatofitoses and ectoparasites (fleas, lice, mallowpha, cheyettella, sarcoptic, and demodectic mange). Cytological examination confirmed surface, superficial or deep pyoderma caused by G+ cocci.

The control group consisted of healthy, mongrels/mixed-breed dogs (five non-pregnant females and five males), aged 1-3 years (average age of dogs was 2.4 years). The dogs were dewormed and vaccinated prophylactically against distemper, canine parvoviriosis, and rabies. The results of clinical examination and dermatological and intradermal tests were negative.

Lymphocyte phenotyping. Flow cytometry was used to measure percentage of blood cells expressing MHC II, CD3, CD4, CD8, and CD21 molecules. Blood samples were collected from the lateral saphenous or cephalic vein to glass, chemically clean and sterile tubes with heparin sodium (Biochemie GmbH, Austria) at 5 mass units/mL of blood. Cytometric monoclonal antibodies (AbD Serotec) directed against surface antigens present on peripheral blood lymphocytes of dogs containing MHC class II molecules (MCA1044F), CD3 (MCA1774F), CD4 (MCA1038F), CD8 (MCA1039F), and CD21 (MCA1781PE) were used. Cytometric analysis was performed on Epics XL flow cytometer (Epics XL flow cytometer Beckman - Coulter, CH-Werfen Comesa Company, USA) in the Department of Epizootiology and Clinic of Infectious Diseases, University of Life Sciences in Lublin. Prior to the determinations, the quality control and automatic standardisation of cytometric examination were carried using Coulter Flow-Check, Coulter Flow-Set, Compensation Reagents preparations. Half-peak coefficient of variation (HPCV) was verified with the expected value in order to check the stability of optical and flow systems. Using the Flow-Set formulation, QuantumTM R-PE MESF (AbD Serotec) and QuantumTM FITC MESF (ABD Serotec) light scattering and fluorescence intensity were standardised by adjusting the tension and the strengthening of a particular item based on the average control application. Reagents were also used to determine the colour levelling system. Designations were made in accordance with the manufacturer's procedure.

Determination of serum IL-10 levels. To determine serum concentration of IL-10, indirect ELISA Quantikine kit with canine IL-10 (R & D Systems) was used. Determination of IL-10 concentration was made in accordance with the procedure recommended by the manufacturer, and the results were expressed in pg/mL.

Statistical analysis. The results were statistically analysed using Statistica 9.3 (StatSoft) and Excel (Microsoft 2007). In order to calculate statistically significant differences between groups, U Mann Whitney test for nonparametric data was performed, whereas the correlations between the parameters studied were determined using rang Spearman's test. P < 0.05 was considered as significant.

Results

The resulting average value (X), standard deviation (± SD), minimum (Min), maximum (Max), and median value (median) of the percentage of MHC II, CD3, CD4, CD8, and CD21 lymphocyte antigens and IL-10 serum concentration in dogs with AD complicated by purulent inflammation of the skin and in control dogs are presented in Table 1.
Table 1. Comparison of the percentage of lymphocytes in peripheral blood of dogs with complicated stage of AD and control group by the U Manna Whitney test

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MHC II (%)</th>
<th>CD3 (%)</th>
<th>CD4 (%)</th>
<th>CD8 (%)</th>
<th>CD21 (%)</th>
<th>IL-10 (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dogs with complicated AD (n = 51)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Min</td>
<td>22.7</td>
<td>38.9</td>
<td>17.1</td>
<td>9.2</td>
<td>12.5</td>
<td>11</td>
</tr>
<tr>
<td>Max</td>
<td>82.5</td>
<td>68.4</td>
<td>46.8</td>
<td>32.5</td>
<td>35.6</td>
<td>54</td>
</tr>
<tr>
<td>Mediana</td>
<td>63.45</td>
<td>58.4</td>
<td>32.5</td>
<td>19.7</td>
<td>20.7</td>
<td>21</td>
</tr>
<tr>
<td>X ±SD</td>
<td>61.7 ± 15.8</td>
<td>57.2 ± 7.7</td>
<td>28.7 ± 7.8</td>
<td>20.1 ± 4.4*</td>
<td>21.5 ± 3.8*</td>
<td>23.8 ± 9.6*</td>
</tr>
<tr>
<td>Control group (n = 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Min</td>
<td>53.4</td>
<td>51.5</td>
<td>21.1</td>
<td>13.7</td>
<td>14.4</td>
<td>6</td>
</tr>
<tr>
<td>Max</td>
<td>65.8</td>
<td>62.1</td>
<td>37.4</td>
<td>15.2</td>
<td>18.5</td>
<td>30</td>
</tr>
<tr>
<td>Mediana</td>
<td>62.2</td>
<td>56.6</td>
<td>26.9</td>
<td>14.0</td>
<td>16.15</td>
<td>12</td>
</tr>
<tr>
<td>X ±SD</td>
<td>60.5 ± 4.3</td>
<td>56.6 ± 4.1</td>
<td>27.4 ± 4.03</td>
<td>14.3 ± 0.6*</td>
<td>16.2 ± 1.5*</td>
<td>14.7 ± 7.7*</td>
</tr>
</tbody>
</table>

* - statistically significant differences

The obtained results showed that in dogs suffering from AD complicated by purulent dermatitis, the average values of lymphocytes with MHC II were 61.7%, CD3 - 57.2%, CD4 - 28.7%, CD8 - 20.1%, and B cells displaying lymphocytes with CD21 molecule - 21.5%. Thus, the ratio of CD4:CD8 was 1.43. An average serum concentration of IL-10 in these dogs was 23.8 pg/mL. By contrast, in the control group, an average percentage of lymphocytes with MHC II was 60.5, CD3 - 56.6, CD4 - 27.4, CD8 - 14.3, and CD21 - 16.2. In this group of dogs, the ratio of CD4:CD8 was 1.92. The mean concentration of IL-10 in the control group was 14.7 pg/mL. The comparative analysis of results obtained in dogs with AD and control group using Mann Whitney U test showed that the percentage of CD8 cells (P = 0.000032) and CD21 cells (P = 0.000046), and concentration of IL-10 (P = 0.004763) were significantly higher in dogs with complicated AD. There were no significant differences in the percentage of MHC class II molecules, CD3, and CD4 count between two groups of dogs.

Spearman’s test analysis of correlations between the examined indicators of immune response displayed a strong positive correlation between class II MHC molecules and CD3 lymphocytes (R = 0.638), between the MHC class II and CD4 lymphocytes (R = 0.596), MHC class II and CD21 cells (R = 0.419), and a negative correlation between class II MHC molecules and CD8 lymphocytes (R = -0.393) in dogs with complicated AD. There was also a strong positive correlation between CD3 and CD4 lymphocytes (R = 0.708), between CD3 and CD21 cells (R = 0.479) and a weaker correlation between CD4 and CD21 (R = 0.385). No correlation was found between IL-10 and other examined indicators. Some selected correlations are shown graphically in Figs 1, 2, 3, and 4. Using Spearman’s test, no correlations were found between examined indicators of immune response in the control dogs.
The percentage of CD4 was 40% in samples of inflammatory cells (8). However, according to Faldyna et al. (6) in healthy adult dogs, this ratio should vary from 1.7 to 2.8. However, according to other authors (15), in healthy dogs the ratio is lower and varies, regardless of age and gender, from 1.5 to 1.7, which is contrary to the previously published data (4, 6).

Comparative analysis of the results received from dogs with AD complicated by purulent dermatitis and healthy dogs showed significant differences between two groups of animals. In dogs suffering from complicated purulent dermatitis a significantly higher percentage of CD8 lymphocytes, as well as significantly higher concentration of serum IL-10 were observed in comparison to healthy dogs. This indicates that at this stage of the disease, in addition to humoral factors, immunosuppression induced by the activity of the infectious agent plays an important role. Literature data indicate that CD8 lymphocytes are responsible for the immunoregulation of effector functions of immunologically competent cells (8). In dogs with AD, they prevent systemic tissue damage as a result of persistent inflammation induced by bacterial infection. An increase in the percentage of CD8 T lymphocytes in dogs with complicated AD compared to healthy dogs caused a decline in the CD4:CD8 ratio to the value of 1.45. This is probably connected with an increase in adhesion capacity, chemotaxis, and diapedesis of inflammatory cells (8). However, the results of research carried out on German Shepherds suffering from deep pyoderma showed that the percentage of CD8 rate (28.6%) did not differ significantly from the results obtained in healthy dogs, while the percentage of CD4 was significantly lower than in healthy dogs (3). Thus, in dogs with deep pyoderma the authors obtained the ratio of CD4:CD8 = 1.3, which was explained as the immunosuppressive effect of CD8 lymphocytes on other lymphocyte subpopulations. In the present study, there was no statistical significance in the percentage of CD4 lymphocytes between healthy dogs and those with AD, but there were significantly higher values of lymphocyte CD21 and IL-10 concentrations in dogs with AD, which confirms the participation of the humoral mechanisms dependent on B lymphocytes in the immune response. Recently published data (9, 17) indicates that high concentration of IL-10 observed in dogs with AD points at its regulatory functions associated with activation of the regulatory lymphocytes.

The lack of variation in the percentage of MHC II, CD4, and CD3 between dogs suffering from AD complicated by purulent dermatitis and healthy dogs may be connected with an increase in the percentage...
of CD8 cells and their immunoregulatory function in the process of antigen presentation, besides the presence of immunosuppressive agents released during bacterial infection. CD4 lymphocytes, which, together with "professional" APC antigen presenting cells, play a central role in the immunoregulation, identify epitopes targeted by the immune response. In response to the antigen, the differentiation of CD4 T cells toward the Th1 profile, which produces IL-2, IL-12, TNF, IFN, or Th2 producing IL-4, IL-5, IL-10, and IL-13 takes place (5, 9, 10, 17, 18). Both T-cell subsets have CD4 surface molecules and recognise the antigen presented in association with MHC class II molecules (5). As the profile of cytokines released by Th1 and Th2 cells act antagonistically to each other, it is assumed that the subpopulation of Th1 cells determines the activity of immune mechanisms in allergic diseases and is involved in delayed-type hypersensitivity mechanisms. It is believed that the sharp change in allergen-induced cytokine profile is the result of stimulation of Th2 lymphocytes, and in chronic changes, the advantage of Th1 profile is marked, however, not much is known about the role of CD8 lymphocytes in this process (17). A statistically significant increase in the B lymphocytes expressing CD21 molecules and a significant increase in the IL-10 concentration in serum observed in dogs with complicated AD confirm the Th2 lymphocyte activation. A significantly higher percentage of CD8 lymphocytes noted in the study suggests that the cellular suppressive mechanisms of the immune response are involved in this stage of the disease, which was previously suggested by Uchida et al. (20). A highly positive correlation between MCH II, lymphocytes CD3, CD4, and CD21, and a negative one between MHC class II molecules and CD8 lymphocytes, found in the study, support this hypothesis. The results of the present study indicate, that, the participation of both humoral and cellular immunologic mechanisms are involved in dogs with complicated AD and this confirms the observations of other authors (1, 5, 9, 11, 17, 20).

The presented results suggest that a number of interactions of factors deepening an already existing immune dysfunction are observed in dogs with AD complicated by purulent dermatitis. Understanding the immune mechanisms leading to worsening of inflammatory and pruritic processes in the course of the disease will establish rules preventing the development of dermatoses complicating AD in dogs.

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References


