NOTE Immunology

CD56 is Expressed Exclusively on CD3+ T Lymphocytes in Canine Peripheral Blood

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ABSTRACT. CD56+ cells in canine blood leukocytes were characterized by flow-cytometric analysis of peripheral blood of 30 healthy adult beagle-dogs (15 males and 15 non-pregnant females). In 19 of the 30 dogs, anti human CD56 antibody, Leu-19, reacted with 8.8–21.7% of peripheral blood lymphocytes. All CD56+ cells simultaneously expressed CD3 molecules on their surface. Further phenotypic analysis revealed that 50.6 ± 13.1% of the CD56+ cells showed CD4+CD8− phenotype and 43.7 ± 10.1% showed CD4−CD8+ phenotype. Expression intensity of CD56 on the CD4+CD8−CD56+ cells was significantly higher than that on CD4−CD8+CD56+ cells (P<0.001). These findings indicate that CD56, which is a neural cell adhesion molecule, is uniquely expressed on subsets of T lymphocytes in canine peripheral blood.

KEY WORDS: canine, N-CAM, natural killer.

CD56, which is a neural cell adhesion molecule (N-CAM), is a membrane glycoprotein belonging to the immunoglobulin superfamily [3, 8]. CD56 is expressed mainly on natural killer (NK) cell in human peripheral blood and is used as a surface marker of human NK cell [2]. Although the precise functional role of CD56 molecules on human NK cells is not fully understood, CD56 is thought to be involved in cell-attachment of NK cells to the target cells in their cytokilling process [10].

In rhesus monkeys, however, peripheral blood NK cells predominantly show CD56− phenotype [1]. Furthermore, CD56 is expressed on almost all peripheral blood monocytes in cynomolgus monkeys in rhesus monkeys [1]. Therefore, CD56 is not always a specific marker for NK cells in mammals other than humans.

An anti human CD56 antibody, Leu-19 (Becton Dickinson, San Jose, California, U.S.A.), cross-reacts with canine leukocytes [14]. However, detailed characteristics of canine CD56+ cells remain unclear.

In this study, we clarified the phenotypic characteristics of canine CD56+ cells by flow-cytometric analysis on canine peripheral blood leukocytes.

Thirty clinically healthy beagle-dogs (15 males and 15 non-pregnant females) 1.4 to 7.3 years old were used in this study. By vaccination, all dogs were free from rabies virus, canine distemper virus, canine parainfluenza virus, and canine parvovirus. Blood was collected from each dog’s jugular vein, using sodium citrate as an anti-coagulant. We confirmed that the complete blood counts and the leukocyte differential counts of all blood samples were within normal ranges (data not shown).

Direct staining of peripheral blood leukocytes was done as follows. The following pairs of antibodies were reacted with 50 µl of samples of blood for 15 min at room temperature: anti human CD56 phycoerythrin conjugated (−PE) antibody (Leu-19; Becton-Dickinson) and anti canine CD3 fluorescein isocyanate conjugated (−FITC) antibody (CA17.2A12; Serotec, Oxford), anti human CD21-PE antibody (B-ly4; Becton-Dickinson) and anti canine CD3-FITC antibody, or isotypic control-FITC antibody (Beckman-Coulter, Fullerton, California, U.S.A.) and isotypic control-PE antibody (Beckman-Coulter). To each of these samples, we added 1.5 ml of Erythrocytes Lysing Buffer (0.83% ammonium chloride, 0.1% potassium hydrocarbonate, and 0.004% EDTA disodium salt in distilled water), and then left the sample for 7 min at room temperature. After centrifugation, the supernatant was removed by aspiration. The pellet was washed twice with wash buffer (phosphate buffered saline containing 0.5% bovine serum albumin, 2 mM EDTA disodium salt, and 0.03% sodium azide) at 4°C. The samples were fixed with 1% paraformaldehyde-phosphate buffered saline, and then subjected to flow-cytometric analysis.

Indirect three-color staining was performed as follows. The following antibodies were reacted with 50 µl of samples of blood for 15 min at room temperature: anti human CD56 unconjugated mouse-antibody (Leu-19; Becton-Dickinson), anti canine CD44-FITC rat-antibody (YKIX302.9; Serotec, Oxford, U.K.), and anti canine CD8α-PE rat-antibody (YCATE55.9; Serotec). Incubation, lysing erythrocytes, and washing were done as described above. To each sample, we added anti murine IgG, biotinylated rat-antibody (A85–1; Becton-Dickinson) and incubated the sample for 10 min on ice. The resulting sample was washed with wash buffer (described above), and then reacted with R-Phycocerythrin-cyanine-5 conjugated streptavidin (C0050; Dako, Japan, Kyoto, Japan) for 10 min on ice. The sample was washed again, and then fixed as described above. To determine the background of the indirect three-color staining, we stained each blood sample with unconjugated isotypic control mouse IgG1 (679.1Mc7; Beckman-Coulter), anti CD4-FITC rat-antibody, and anti CD8α-PE rat-antibody in the
same method.

The fluorescence of the stained cells was detected by using a FACS Calibur (Becton-Dickinson) and was analyzed by using the commercially available software CellQuest (Becton-Dickinson), as described previously [12]. Student's t-test was used to determine statistical significance.

We separated lymphocytes from monocytes-granulocytes by gating on the forward scatter and side scatter histogram as described in detail elsewhere [4]. The results show that the proportion of each lymphocyte-subset was as follows, given as mean and standard deviation (SD) for all 30 dogs: CD3+ cells were 85.1 ± 6.8%, CD21–CD3– cells were 12.0 ± 7.1%, and CD21–CD3+ cells were 2.9 ± 1.1%. In 19 of the 30 dogs, the anti CD56 antibody, Leu-19, reacted with 8.8–21.7% of peripheral blood lymphocytes, whereas the other 11 dogs had no Leu-19+ cells on the lymphocytes-gate (Fig. 1). None of the 30 dogs had any CD56+ cells on the monocytes-granulocytes-gate (data not shown).

Two-color flow-cytometric analysis revealed that almost all the CD56+ cells in the reactive 19 dogs expressed CD3 molecules on their surface (Fig. 2) and that the CD56+ cells occupied 16.4 ± 5.3 (mean ± SD, n=19)% of CD3+ lymphocytes.

Three-color flow-cytometric analysis, which is detectable for CD56, CD4, and CD8 molecules simultaneously, revealed that 50.6 ± 13.1 (mean ± SD, n=19)% of the CD56+ cells were phenotypically identified with CD4–CD8+ subsets and 43.7 ± 10.1 (mean ± SD, n=19)% with CD4+CD8– subsets (Fig. 3). Comparison of the expression intensity of CD56 between the subsets shows that the geometric mean fluorescence of CD56 in CD8– cells (334.0 ± 118.1) was significantly higher (P<0.001) than that in CD4+ cells (160.4 ± 54.2) (Fig. 4).

Our study shows that CD56 was expressed on subsets of CD3+ T lymphocytes in canine peripheral blood, and that canine peripheral blood CD56+ cells consisted of at least two subsets, namely, CD3+CD4+CD8+CD56+(high) cells and CD3+CD4–CD8+CD56+(low) cells.

In humans, the peripheral blood CD56+ cells are predominantly CD3–CD56+ phenotype occupying 1–22% of lymphocytes, with only a relatively minor amount of CD3+CD56+ phenotype (0–6%) [11]. Therefore, expression of CD56 in dogs clearly differs from that in humans.

Norris et al. reported that CD3+CD56+ cells, called natural T cells, were numerous in the human liver (from 13.5 to 54.7% of lymphocytes) [11]. Natural T cells are reportedly involved in innate immunity and immunoregulation [11], and are modulated in salmonellosis and in the infection of human immunodeficiency virus [6]. It is still unknown if canine CD3+CD56+ cells are functionally identical with the human CD3+CD56+ cells. CD56 is a member of cell-adhesion molecule called N-CAM, therefore, CD56 molecules of canine peripheral blood lymphocytes must be considered to be involved in cell-attachment of the subpopulation of CD4+ and CD8+ T lymphocytes.

In our experiments, 11 out of the 30 dogs had no cells that were detected by the anti-CD56 antibody, Leu-19 (Fig. 1). Uda et al. reported that heterogeneity around an epitope on a surface protein, CD3, of T-lymphocytes of cynomolgus monkey can cause diverse reactivity of a monoclonal antibody, FUN-1, within the species [13]. The epitope of Leu-19 is in the third immunoglobulin-like domain of the extracellular portion of human CD56 [5]. It is still unknown...
whether the lymphocytes of the 11 dogs expressed another form of CD56 that could not be recognized by Leu-19 because of their supposed polymorphism around the epitope or did not express any CD56 molecule on their surface.

The reactivity of Leu-19 to canine CD56 was also evidenced by the immunohistological examination using several canine tissues (i.e., cervical spinal cord, adrenal gland, and pancreas). In those tissues, white and gray substance of spinal cord, adrenal medulla, islets, and unmyelinated peripheral nerve fibers were positively immunostained by Leu-19 (data not shown) and the staining pattern was similar to the distributions reported in the study on rat CD56 [7].

In this study, we demonstrated that CD56 molecules expressed exclusively on CD3⁺ T lymphocytes in canine peripheral blood leukocytes. The immunological role of CD56 in canine T lymphocytes needs further study.

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