Expression of Kit, the Receptor for Stem Cell Factor, in Bovine Peripheral Blood

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ABSTRACT. The expression of Kit, the receptor for stem cell factor (SCF), on bovine peripheral blood cells (PBCs) was examined by using monoclonal antibodies against the bovine Kit protein. Flow cytometric analysis showed that approximately 1.5% of PBCs expressed Kit. In cytopsin preparations, the morphology of most Kit+ PBCs was similar to that of large lymphocytes. Subsets of Kit+ PBCs co-expressed CD3, IgM, and/or CD11b but not CD14 or G1. SCF did not induce the proliferation of Kit+ PBCs. These results indicate that Kit is expressed on subsets of lymphocytes in bovine peripheral blood, but the ligand of Kit, SCF, does not directly induce the proliferation of this cell population.

KEY WORDS: bovine, Kit receptor, stem cell factor.

NOTE Immunology

The receptor Kit is a transmembrane tyrosine kinase encoded by the proto-oncogene c-kit [21]. Stem cell factor (SCF), the ligand of Kit, exerts its biologic effects by binding to and activating Kit on the surface of target cells [8]. Therefore, determining the expression and distribution of Kit is a first step to understanding the function of this receptor and its ligand, SCF.

Kit is expressed in the hematopoietic progenitor/stem compartment of bone marrow (BM), and SCF is involved in early stages of hematopoiesis [1, 12]. In humans, Kit is expressed predominantly on immature bone marrow cells (BMCs) that have a blast-like morphology [2, 15]. When combined with various hematopoietic cytokines, SCF induces Kit+ BMCs to form colonies in semisolid culture [15].

In addition, Kit is expressed on a minor subset of mature cells in peripheral blood (PB), and SCF plays various roles in these cell populations [12]. In humans, Kit is expressed on a small subset of CD56+ NK cells, which represent fewer than 1% of the total peripheral blood mononuclear cells (PBMCs) [13]. [3H]Thymidine incorporation assays show that SCF does not directly induce the proliferation of CD56+ NK cells; rather, SCF enhances IL-2-induced proliferation of this cell population [13]. Kit is expressed on eosinophils at low intensity, and SCF stimulates the binding of eosinophils to adhesion molecules such as fibronectin [22]. Further, Kit is expressed on basophils at low intensity, and SCF enhances IgE-dependent histamine release from these cells [4].

To fully understand the role of Kit and SCF in cattle, determining the expression and distribution of Kit in the target animal is needed because the composition of the bovine immune system differs somewhat from that in humans [5]. Our recent study in cattle showed that Kit was expressed predominantly on BMCs with a blast-like morphology, and SCF induced Kit+ BMCs to form colonies in semisolid culture [9]. These results suggest that Kit and SCF play an important role in the early stages of hematopoiesis in bovine BM as they do in human BM. However, the expression and function of Kit in bovine PB have not been examined previously.

In the present study, we used flow cytometry to examine the expression and distribution of Kit in bovine PB. We then assessed the proliferative capability of Kit+ BMCs in the presence of SCF by using [3H]thymidine incorporation and colony formation assays to determine whether SCF directly induces the proliferation of Kit+ BMCs, as it does in Kit+ BMs.

Flow cytometric analysis. We incubated whole PB (100 µl) from each of six adult female Holstein-Friesian cows with a biotinylated anti-bovine Kit monoclonal antibody (mAb; bK-2 [9]; 10 µg/ml; IgG2a) for 30 min on ice. After washing the cells with 3 ml phosphate-buffered saline (PBS) containing 1% fetal calf serum (FCS), we incubated them with phycoerythrin (PE)-conjugated streptavidin (Coulter Immunotech, Marseille, Cedex, France) for 30 min on ice. Erythrocyte-lysing solution (154 mM NH4Cl, 10 mM KHCO3, 0.082 mM EDTA-4Na) then was added to remove red blood cells. After washing, the cells were resuspended in PBS containing 1% FCS and 2 µg/ml propidium iodide (PI) and then analyzed by using a flow cytometer (Epics Elite, Coulter, Hialeah, FL, U.S.A.). By using control cells comprising peripheral blood cells (PBMCs) that had been stained with a biotinylated unrelated control mAb (Dako, Glostrup, Denmark; IgG2a) and PE-conjugated streptavidin, we defined thresholds for distinguishing positively stained cells from negatively stained ones [11]. For morphologic analysis of Kit+ BMCs, we used a fluorescence-activated cell sorter (FACS; Epics Elite, Coulter) to sort Kit+ BMCs from whole PB. We then used the sorted cells in cytopsin (Shandon, Runcorn, Cheshire, UK) preparations, which we stained with Giemsa (Muto Pure Chemicals, Tokyo, Japan).

Two-color flow cytometric analysis was performed to further characterize Kit+ BMCs. PBMCNs were fractionated from whole PB by using Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden) density-gradient centrifugation according to the manufacturer’s recommendations. We stained the PBMCNs with anti-bovine Kit mAb (bK-2) and sorted for...
Kit+ PBCs by using a FACS. We incubated the Kit+ PBCs with a mAb against a bovine lineage marker (CD3 [6], IgM [19], CD11b [17], CD14 [18], or G1 [14]; VMRD, Pullman, WA, U.S.A.; 10 µg/ml; IgG1) for 30 min on ice. After washing, we incubated the cells with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG1 (Southern Biotechnology Associates, Birmingham, AL, U.S.A.) for 30 min on ice. After washing, the cells were resuspended in PBS containing 1% FCS and PI and then analyzed by using a flow cytometer.

[3H]thymidine incorporation assay: We stained PBMNCs with a biotinylated anti-bovine Kit mAb (bK-1 [9]; 10 µg/ml; IgG1) and PE-conjugated streptavidin then sorted the cells into Kit+ and Kit– fractions by using a FACS. Sorted cells (1 × 10^4) were cultured in 200 µl Iscove’s modified Dulbecco’s medium (IMDM) containing 10% FCS and recombinant bovine SCF (25 ng/ml) [9, 23] in 96-well plates (Falcon 1177, Becton Dickinson, Franklin Lakes, NJ, U.S.A.) at 37°C in a humidified atmosphere of 5% CO2. Using unsorted BMCs, we previously established that this concentration of SCF induced the maximum [3H]thymidine uptake (data not shown). The cells were radiolabeled with 1 µCi [3H]thymidine (20 Ci/mmol; New England Nuclear, Boston, MA, U.S.A.) during the last 6 hr of the 48-hr culture period. The labeled cells were harvested on glass-fiber filters, and the incorporated radioactivity was measured by using a liquid scintillation counter (Beckman, Fullerton, CA, U.S.A.). Thawed cryopreserved bone marrow mononuclear cells (BMMNCs) were similarly stained with bK-1, sorted into Kit+ and Kit– fractions, and examined for [3H]thymidine incorporation.

Colony formation assay: Colony formation assays were performed as described previously [9]. Briefly, Kit+ PBMNCs (1 × 10^5) were cultured in 1 ml semisolid IMDM containing 1% methylcellulose and recombinant bovine SCF (100 ng/ml). We counted colonies containing more than 50 cells on day 7 by using an inverted microscope.

We found that 1.5% ± 0.7% (mean ± standard deviation for data from six animals) of all PBCs expressed Kit. This staining seemed to be specific because preincubation of cells with an excess of another anti-bovine Kit mAb (bK-5), which recognizes the same (or a closely related) epitope as that recognized by bK-2 [9], abrogated this staining, but preincubation with an unrelated mAb did not. Kit+ PBCs showed low forward and side light-scattering characteristics, similar to those of peripheral CD3+ lymphocytes (Fig. 1). The morphology of Giemsa-stained cytospin preparations of bovine Kit+ PBCs is similar to that of large lymphocytes.
THE KIT RECEPTOR IN BOVINE PERIPHERAL BLOOD

for data from four animals), IgM (B cells, 16.4% ± 10.9%), and/or CD11b (NK cells and myeloid cells, 82.7% ± 11.8%), but no Kit+ cells significantly expressed CD14 (monocytes, 0.8% ± 0.6%) or G1 (granulocytes, 0.7% ± 0.4%; Fig. 3). In [3H]thymidine incorporation assays, neither Kit+ nor Kit− PBMNCs proliferated in the presence of SCF, whereas Kit+ BMMNCs showed SCF-dependent proliferation under the same conditions (Fig. 4). Kit+ and Kit− PBMNCs in the presence of SCF failed to form colonies in semisolid culture (experiments were repeated three times).

We showed that the receptor Kit was expressed on subsets of PBCs with lymphocyte-like morphology. These Kit+ PBCs were positive for CD3, IgM, and/or CD11b. Unlike in human PB, neither bovine eosinophils nor basophils expressed Kit. These results suggest that Kit is expressed on subsets of lymphoid lineage cells in bovine PB. It is interesting to note that in bovine BM, Kit is expressed not only on lineage marker-negative cells with a blast-like morphology but also on CD3+ cells with a lymphocyte-like morphology [10]. In humans, CD3+ BMCs do not express Kit [3], and Kit expression is restricted to a small subset of NK cells in PB [13] and to early T cell precursors in the thymus [7]. In short, Kit expression in the lymphoid lineage cells of cattle differs from that in humans, suggesting that SCF may have a functional role in Kit+ bovine lymphocytes and that an equivalent role has yet to be identified in humans.

We examined whether SCF directly induced the proliferation of Kit+ PBCs because SCF is involved in the prolifer-
ation of and colony formation by Kit+ BMCs in cattle [9] and humans [15]. In addition, one of the key functions of tyrosine kinase receptors and their ligands is the induction of cell proliferation in many cell types [20]. However, we found that Kit+ PBCs, unlike Kit+ BMCs, failed to proliferate and form colonies in the presence of SCF in vitro. In humans, SCF alone has no effect on the proliferation of Kit+ NK cells but enhances the IL-2-induced proliferative response of this cell population [13]. Similarly, neither SCF nor IL-3 induces colony formation by Kit+ mast cell progenitors in murine fetal PB, but these factors act synergistically to induce this cell population to form colonies in semisolid culture [16]. Therefore, when in the presence of another hematopoietic cytokine or cytokines, SCF may enhance or induce the proliferation of bovine Kit+ lymphocytes.

In conclusion, we have shown that the receptor Kit is expressed on subsets of lymphocytes in bovine peripheral blood, but its ligand, SCF, fails to induce directly the proliferation of Kit+ PBCs.

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REFERENCES