Identification of Exocytosis Mediator Proteins in Peripheral Blood Neutrophils of Patients with Chronic Myeloid Leukemia

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We demonstrated expression of plasma membrane proteins (syntaxin-4 and syntaxin-6) and specific/gelatinase granule membrane proteins (SNAP-25 and VAMP-2) in the peripheral blood neutrophils of patients with chronic myeloid leukemia. VAMP-1 associated with membranes of azurophilic and specific/gelatinase granules was absent in peripheral blood neutrophils of patients with chronic myeloid leukemia. Decreased capacity of neutrophils to exocytosis in chronic myeloid leukemia is probably caused by the absence of VAMP-1 in these cells.

Key Words: *neutrophil; chronic myeloid leukemia; exocytosis; SNARE*

Chronic myeloid leukemia (CML) is a myeloproliferative disease resulting from malignant transformation of the stem hemopoietic cell and characterized by cell capacity to differentiation and maturation and by enhanced production of granulocytes (primarily neutrophils) [1,2]. Previous studies revealed morphological and functional defects in the peripheral blood neutrophils of CML patients: decreased chemotaxis, adhesion, bactericidal activity, secretion and activities of some enzymes of intracellular granules, and expression of certain differentiation antigens [1,2,5,8,15].

Realization of the key functions of neutrophils (defense and inflammatory) largely depends on exocytosis of specialized intracellular granules [4]. On the other hand, it is known that exocytosis in neutrophils is realized through a universal mechanism of membrane adhesion/fusion SNARE-dependent mechanism. This mechanism is based on the interaction of vesicular membrane protein VAMP (vesicle-associated membrane protein, v-SNARE) with plasma membrane proteins (t-SNARE) syntaxin and SNAP-25 (synaptosome-associated protein, molecular weight 25 kDa), formation of complexes (SNARE complex), binding of cytosol proteins to this complex, and, finally, membrane fusion [7]. Human peripheral blood neutrophils contain various isoforms of SNARE group proteins, which probably mediate exocytosis of granules of different types [6,10,12]. This paper presents data on immunodetection of individual SNARE proteins in peripheral blood neutrophils of CML patients.

MATERIALS AND METHODS

Neutrophils of CML patients (full-developed disease with typical clinical and hematological symptoms) and donors were isolated from fresh peripheral blood by erythrocyte sedimentation with dextran, centrifugation in Ficoll Hypaque gradient, and subsequent hypertonic lysis of erythrocytes [11]. Equal volumes of isolated cells were destroyed in 20 mM tris-HCl buffer (pH 7.2) containing: 100 mM KCl, 0.9% Triton X-100, 10% glycerol, 2 mM phenylmethane sulfonylfluoride. After centrifugation (2000*g*, 20 min, 4°C) the supernatant (equal volumes) was subjected to electrophoresis in 12% PAAG [9] and immunoblotting [14] with SNARE-recognizing antibodies. Monoclonal antibodies to syntaxin-4 (Transduction Laboratories), VAMP-2 (Synaptic Systems), SNAP-25 (Stemberger Monoclonals), and VAMP-1-recognizing poly-

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clonal antibodies (Synaptic Systems) were used. Monoclonal antibodies 3D10 to syntaxin-6 were kindly provided by Dr. H. B. Bock from Stanford University, USA.

RESULTS

The presence of two isoforms of syntaxin protein family (syntaxin-4 and syntaxin-6) in human peripheral blood neutrophils was previously demonstrated [10]. These proteins are located in the plasma membrane and probably act as t-SNARE during exocytosis [10]. Monoclonal antibodies to syntaxin-4 recognize the only band corresponding to protein with a molecular weight of ~32 kDa in extract of peripheral blood neutrophils from CML patients (Fig. 1, *a*). This immunoreactive protein is characterized by the same electrophoretic mobility as syntaxin-4 detected in the neutrophil extract from donor peripheral blood. The results of immunoblotting with monoclonal antibodies to syntaxin-6 indicate that another representative of the syntaxin family, syntaxin-6 (protein with molecular weight of about 31 kDa) is also present in neutrophil extract from CML patients (Fig. 1, *b*).

It was previously shown that human neutrophils express two VAMP family proteins, VAMP-1 and VAMP-2, associated with membranes of mobilized intracellular granules and presumably participating in

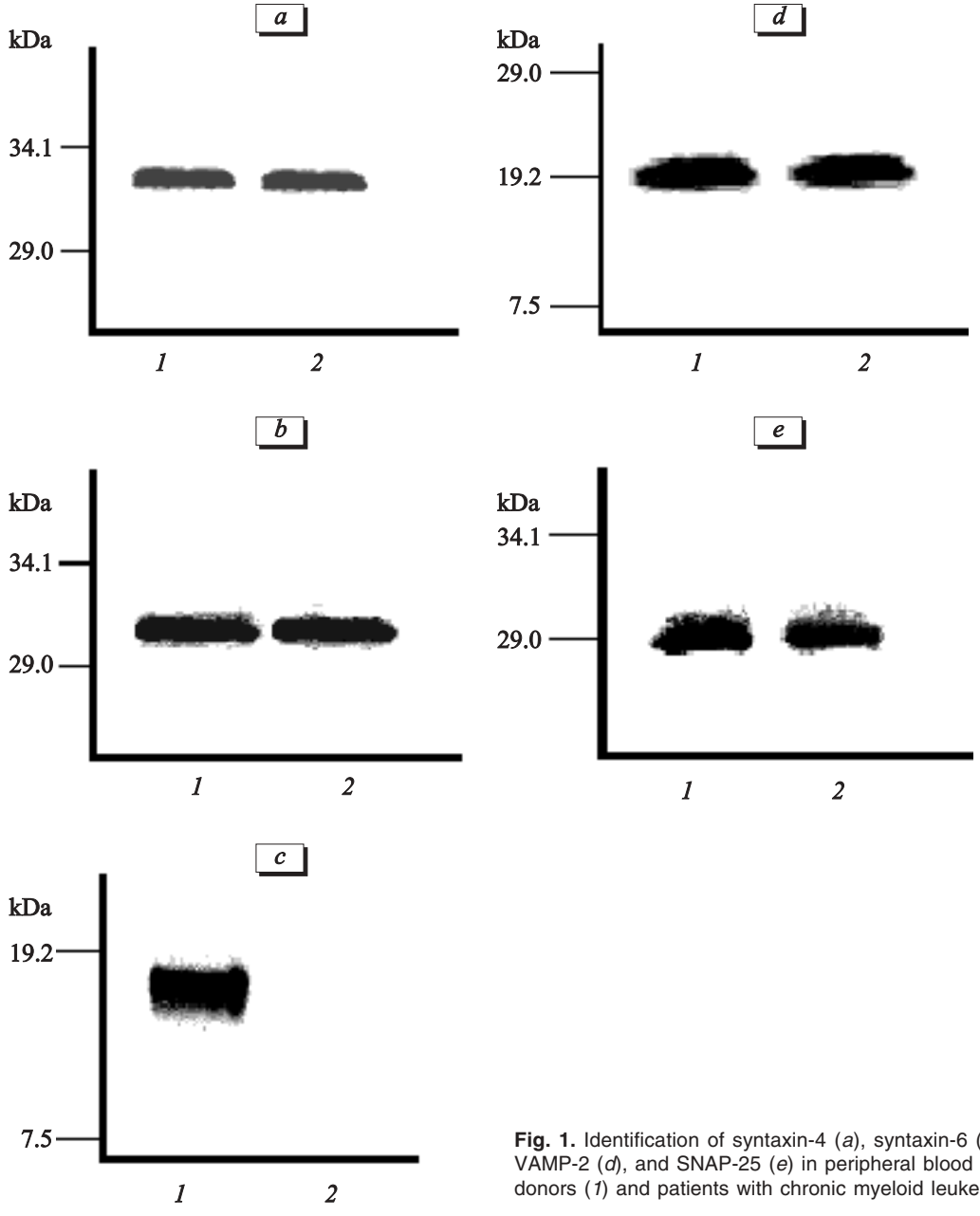

Fig. 1. Identification of syntaxin-4 (*a*), syntaxin-6 (*b*), VAMP-1 (*c*), VAMP-2 (*d*), and SNAP-25 (*e*) in peripheral blood neutrophils from donors (*1*) and patients with chronic myeloid leukemia (*2*).

exocytosis as v-SNARE [3,6]. Immunodetection with VAMP-2-specific antibodies demonstrated an immunoreactive band corresponding to protein with a molecular weight ~18 kDa both in the control and in CML (Fig. 1, *d*). VAMP-1 protein, was not detected in neutrophil extract from CML patients (Fig. 1, *c*).

We previously showed that SNAP-25 protein (the third protein component of the SNARE complex) is present in human neutrophils and associated with membranes of intracellular granules [12]. Immunoblotting showed the presence of SNAP-25 (immunoreactive protein) in neutrophils of CML patients (Fig. 1, *e*).

Hence, our studies showed that peripheral blood neutrophils of CML patients express plasma membrane proteins syntaxin-4 and syntaxin-6 and membrane proteins of specific/gelatinase granules SNAP-25 and VAMP-2. VAMP-1 protein associated with membranes of azurophilic and specific/gelatinase granules in donors was absent in CML patients. Our findings suggest that defects in the exocytosis system of leukemic neutrophils can be determined by the absence of VAMP-1 in these cells. This conclusion is confirmed by published data on association of CML with disorders in the granule formation system leading to quantitative predominance of azurophilic granules [2,13]. On the other hand, abnormal quantitative distribution of SNARE on granules of different types in CML and its contribution to disorders in exocytosis processes cannot be excluded.

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